

91600001 Rev G

# MERSCOPE<sup>®</sup> Instrument

## User Guide



### Vizgen Imaging Consumables

10400004	MERSCOPE 140 Gene Imaging Kit, 1 sample
10400005	MERSCOPE 300 Gene Imaging Kit, 1 sample
10400006	MERSCOPE 500 Gene Imaging Kit, 1 sample

**vizgen<sup>®</sup>**

## NOTICES

Document number	91600001
Document revision	Rev G
Revision date	June 2023

### Legal notices

ALL PRODUCT(S) AND SERVICES DESCRIBED HEREIN ARE INTENDED FOR RESEARCH USE ONLY, AND NOT FOR USE IN DIAGNOSTIC OR TREATMENT PROCEDURES OR FOR ANY OTHER USE.

FAILURE TO COMPLETELY READ AND EXPLICITLY FOLLOW ALL OF THE INSTRUCTIONS CONTAINED HEREIN MAY RESULT IN DAMAGE TO THE PRODUCT(S), INJURY TO PERSONS, INCLUDING TO USERS OR OTHERS, AND DAMAGE TO OTHER PROPERTY. VIZGEN, INC. ("Vizgen") DOES NOT ASSUME ANY LIABILITY ARISING OUT OF THE IMPROPER USE OF THE PRODUCT(S) DESCRIBED HEREIN (INCLUDING PARTS THEREOF OR SOFTWARE) OR ANY USE OF SUCH PRODUCT(S) OUTSIDE THE SCOPE OF THE EXPRESS WRITTEN LICENSES OR PERMISSIONS GRANTED BY VIZGEN IN CONNECTION WITH CUSTOMER'S ACQUISITION OF SUCH PRODUCT(S).

Nothing contained herein shall constitute any warranty, express or implied, as to the performance of any product(s) described herein. Any and all warranties applicable to any product(s) and software are set forth in the applicable terms and conditions of sale accompanying the purchase of such product(s). Vizgen provides no warranty and hereby disclaims any and all warranties as to the use of any third-party product(s) or protocols described herein. The use of product(s) described herein is subject to certain restrictions as set forth in the applicable terms and conditions of sale accompanying the purchase of such product(s).

Updates to product(s) and software may be required to enable customers to use product(s). If you fail to update the product(s) or software when provided by Vizgen, any warranty thereon shall immediately terminate. In the event of a product failure resulting from an update, such failed product will be subject to the warranty set forth in the terms and conditions agreed to in connection with the purchase of such product, if any, only if such product is covered by such warranty at the time of such failure. Vizgen is not required to repair, replace, or take any other remedial action with respect to any product not covered under such warranty.

Vizgen® and MERSCOPE® are trademarks of Vizgen. All rights in the trademarks, service marks, trade dress, logos and copyrights are owned by Vizgen, Inc. and fully reserved. All other brands and names contained herein are the property of their respective owners.

This document and its contents are proprietary to Vizgen, Inc. and its affiliates, and are intended solely for the contractual use of its customer in connection with the use of the product(s) described herein and for no other purpose. This document and its contents shall not be used or distributed for any other purpose and/or otherwise communicated, disclosed, or reproduced in any way without the prior written consent of Vizgen. Vizgen does not convey any license under any patent, trademark, copyright, or common-law rights, nor similar rights of any third parties, by this document. The instructions in this document must be strictly and explicitly followed by qualified and properly trained personnel in order to ensure the proper and safe use of the product(s) described herein. All of the contents of this document must be fully read and understood prior to using such product(s).

© 2023 Vizgen, Inc. All rights reserved.

## WARNINGS



Vizgen laser safety is evaluated according to the IEC 60825 1:2014 and found to be conforming to the class 1M.

Viewing the laser output with telescopic optical instruments, such as binoculars or telescopes, may pose an eye hazard and thus the user should not direct the beam to an area where such instruments are likely to be used.



User should exercise caution in attaching and removing electrical cables only when power is removed from the MERSCOPE Instrument (power is de-energized). The improper use of these cables can result in the potential of electric shock.

For the safety of the user:

- User shall use the Vizgen-supplied main power cable. The use of any other main power cables can result in the malfunction of the instrument and can result in the potential of electrical shock to the users.
- Only authorized Vizgen representatives should uncrate and install the MERSCOPE Instrument. Mishandling of the instrument can affect the alignment or damage instrument components.
- Do not relocate the instrument after installation and preparation. Moving the instrument improperly can affect optical alignment and compromise data integrity. If the instrument must be relocated, contact Vizgen Support ([support@vizgen.com](mailto:support@vizgen.com)).
- Uncrating or moving an instrument by anyone other than an authorized Vizgen representative will void the warranty.
- Never use an extension cord to connect the instrument to a power supply.

## SUPPORT

### Contact information

[support@vizgen.com](mailto:support@vizgen.com)

61 Moulton Street

West Cambridge Science Park

Cambridge, MA 02138

USA

Other Vizgen references are available online at <https://vizgen.com/>

## VERSION CHANGE NOTES

Summary of substantive changes from Rev F to Rev G

Brief description of change	Page(s)
Minor updates to the MERSCOPE Instrument packing lists	11
Additional MERSCOPE Instrument cable connections diagram. Refer to the <i>MERSCOPE Instrument Site Preparation Guide</i> for more information on site preparation	62
Updated environmental conditions limits (temperature). Refer to the <i>MERSCOPE Instrument Site Preparation Guide</i> for more information on site preparation	64

## TABLE OF CONTENTS

<b>INTRODUCTION</b> .....	<b>7</b>
MERFISH Technology.....	7
The Vizgen MERSCOPE Platform Solution .....	7
The MERSCOPE Workflow.....	8
Broad Application .....	8
<b>MERSCOPE INSTRUMENT OVERVIEW</b> .....	<b>9</b>
<b>INSTRUMENT PACKING LISTS</b> .....	<b>11</b>
Vizgen Materials.....	11
<b>IMAGING CONSUMABLES</b> .....	<b>12</b>
Vizgen Materials.....	12
Required Materials and Recommended Suppliers .....	13
<b>MERSCOPE INSTRUMENT IMAGING SUMMARY</b> .....	<b>14</b>
<b>MERSCOPE INSTRUMENT IMAGING STEP-BY-STEP</b> .....	<b>15</b>
Prepare for Imaging .....	15
Prepare for Imaging after an Idle Period .....	16
Stain the MERSCOPE Slide with DAPI and PolyT Stain .....	16
Configure the Experiment – Start MERFISH .....	17
Load – MERSCOPE Imaging Cartridge Activation and Loading .....	19
Load – MERSCOPE Flow Chamber.....	22
Select Regions of Interest .....	27
Switch to the High-Magnification Objective.....	28
Experiment – Data Acquisition .....	30
Segmentation Parameters and Image Processing .....	30
Clean.....	32
Home Page Top Navigation Bar .....	34
<b>INSTRUMENT WASH (MAINTENANCE)</b> .....	<b>35</b>
<b>INSTRUMENT INSTALL AND OPERATION</b> .....	<b>37</b>
<b>INSTRUMENT SHUTDOWN (IDLE) AND RESTART</b> .....	<b>38</b>
<b>MERSCOPE INSTRUMENT OUTPUT FILE STRUCTURE AND FORMATS</b> .....	<b>42</b>
Analysis Output Folder Content .....	42
Region Output Folder Content – Standard .....	45
Region Output Folder Content – Segmentation Dependent.....	46
Region Output Folder Content – Images .....	51
<b>TECHNICAL TIPS</b> .....	<b>52</b>
<b>FRONT PANEL LED INDICATORS</b> .....	<b>54</b>
<b>ERRORS AND WARNINGS</b> .....	<b>55</b>
<b>TROUBLESHOOTING</b> .....	<b>57</b>
<b>APPENDIX A: SAFETY DOCUMENTATION</b> .....	<b>59</b>

## INTRODUCTION

### MERFISH Technology

Multiplexed error-robust fluorescence in situ hybridization (MERFISH) is a spatially resolved single-cell transcriptome profiling technology. MERFISH combines the power of single-cell transcriptomics with spatial biology by directly visualizing and counting RNA transcripts from 100s to >10,000 genes in cells or tissue sections.

MERFISH advances the power of single-molecule fluorescence in situ hybridization (smFISH) – the gold standard of RNA quantification – with error robust barcoding, combinatorial labeling, and sequential imaging to greatly expand the multiplexing capacity. MERFISH enables researchers to map the molecular, cellular, and functional composition of biological systems with spatial context.

### The Vizgen MERSCOPE Platform Solution

The Vizgen MERSCOPE Platform Solution is comprised of the MERSCOPE Gene Panel Design Software, MERSCOPE reagent kits, the MERSCOPE Instrument, the MERSCOPE Analysis Computer, and the MERSCOPE Vizualizer software to streamline the acquisition of high quality MERFISH data and data interpretation.

Reagent kits facilitate sample preparation. The automated MERSCOPE Instrument integrates high-resolution imaging and fluidics to automatically acquire a full MERFISH dataset. The MERSCOPE Vizualizer software automates image processing and offers interactive visualization tools to explore MERFISH data.



## The MERSCOPE Workflow

Begin by either designing a MERSCOPE Custom Gene Panel or ordering a MERSCOPE Predesigned Gene Panel.

**Design a custom gene panel.** The intuitive MERSCOPE Gene Panel Design Software makes recommendations on your custom gene panel for optimal performance in a MERFISH measurement. For example, the software highlights genes that are too short or whose abundance may be too high and could cause optical crowding artifacts. Vizgen is currently offering custom gene panels for up to 140 genes, up to 300 genes, and up to 500 genes.

**Order your MERSCOPE Gene Panel.** MERSCOPE Custom Gene Panels and MERSCOPE Predesigned Gene Panels are delivered in a ready-to-use format. Once the gene panel is finalized, users will be able to download their panel-specific MERSCOPE Codebook to the MERSCOPE Instrument.

**Prepare your sample.** Vizgen's sample preparation user guides step you through sample preparation on MERSCOPE Slides.

**Load and run the MERSCOPE Instrument.** The MERSCOPE Slide is assembled into the MERSCOPE Flow Chamber and then loaded into the instrument along with a MERSCOPE Imaging Cartridge. Users define regions of interest on the MERSCOPE Slide and initiate the fully automated instrument run.

**Data Processing and Visualization.** The MERSCOPE Instrument Software (in combination with the MERSCOPE Analysis Computer) automatically processes the raw images to output spatial genomics measurements in a format ready for immediate downstream analysis. The output includes the list of all detected transcripts and their spatial locations in three dimensions (CSV files), mosaic images (TIFF), experiment metadata (JSON), output from the cell segmentation analysis: transcripts per cell matrix (CSV), cell metadata (CSV), cell boundaries (HDF5 [MERSCOPE Instrument Software v231 or earlier] or PARQUET [v232 or later]), and a binary for use with the MERSCOPE Vizualizer software. The MERSCOPE Platform Solution includes the MERSCOPE Vizualizer software for visualizing and analyzing data. The output files are also compatible with open-source tools for single-cell and spatial analysis.

## Broad Application

The MERSCOPE Platform Solution has broad application in both fundamental biology and medicine – from basic science, to drug discovery, to clinical pathology. Find out more at <https://vizgen.com/applications/>.



Oncology



Immunology



Neuroscience



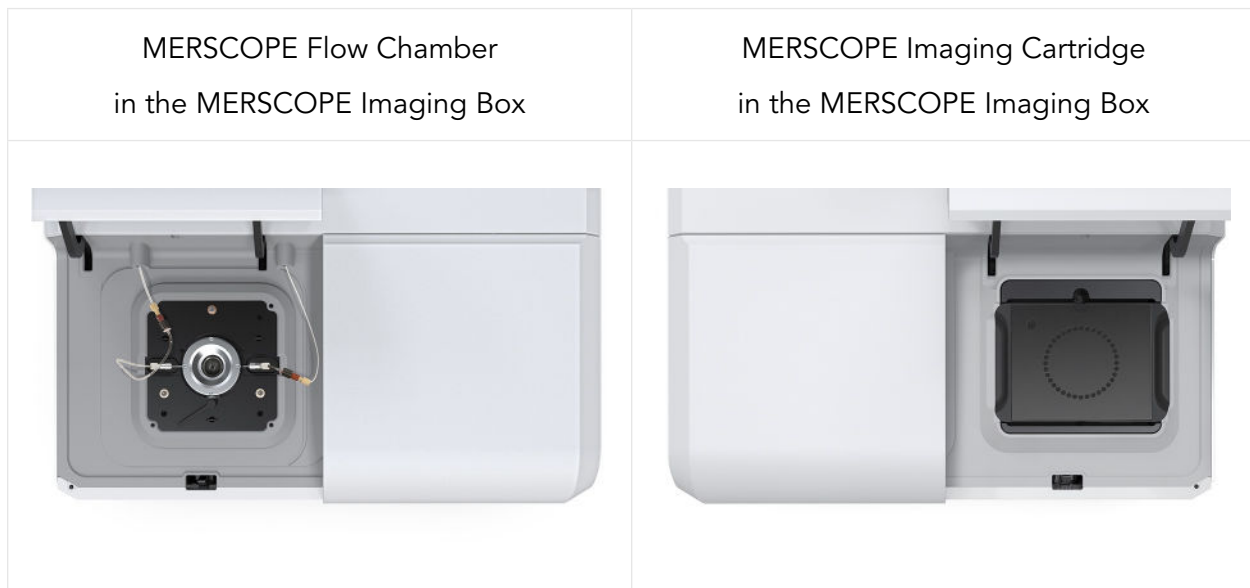
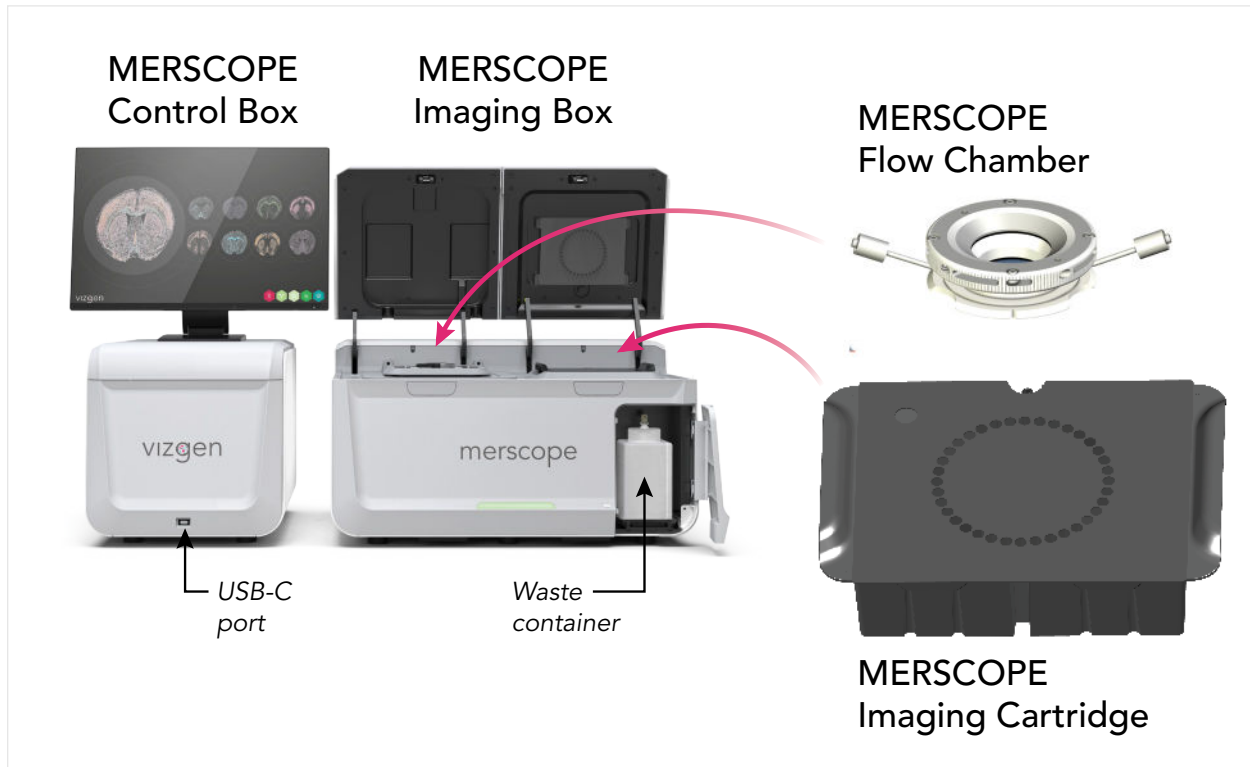
Infectious Disease

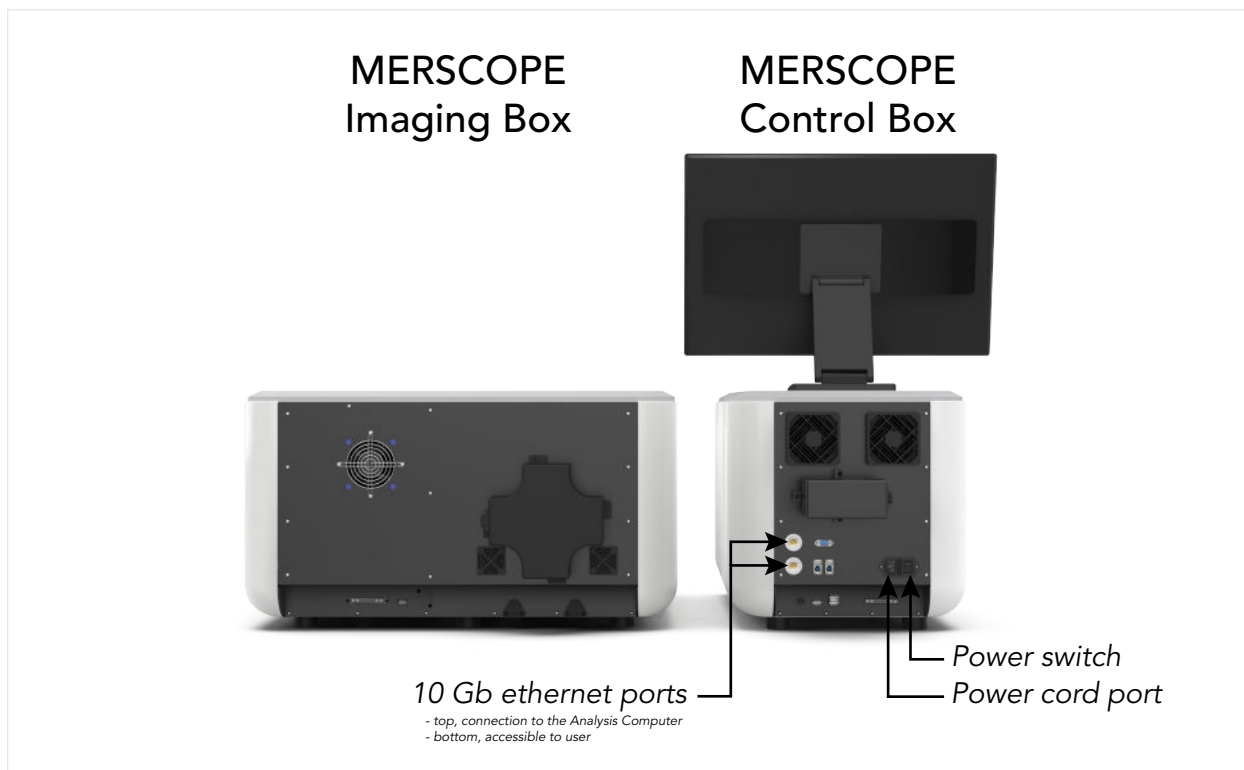


Developmental Biology &  
Regenerative Medicine



## MERSCOPE INSTRUMENT OVERVIEW





The MERSCOPE Instrument is a combination of the MERSCOPE Imaging Box and MERSCOPE Control Box, where the instrument is programmed via an intuitive touchscreen user interface. The MERSCOPE Analysis Computer processes the images acquired on the instrument to generate the list of detected transcripts, mosaic images, and cell segmentation results.

If the MERSCOPE Instrument is not connected to the internet, the applicable MERSCOPE Codebook must be transferred to the instrument in advance of initiating an experiment. The intuitive instrument user interface guides users through experimental setup.

The MERSCOPE Imaging Cartridge is supplied and stored at  $-20^{\circ}\text{C}$  and must be fully thawed before use. When thawed, the imaging cartridge is activated by manual addition of an activation mix, followed by layering with mineral oil. The activated imaging cartridge is then inserted into the instrument.

The MERSCOPE Slide from sample preparation undergoes final preparation on the bench before careful assembly into the MERSCOPE Flow Chamber. The optical surface is cleaned, and it is connected to the MERSCOPE Instrument fluidic lines.

The MERSCOPE Instrument first acquires a low-resolution mosaic. Then, users select the regions of interest for MERFISH imaging, switch the instrument to a high-magnification objective, and start the fully-automated experiment.

## INSTRUMENT PACKING LISTS

### Vizgen Materials

MERSCOPE Instrument	1000001
MERSCOPE Imaging Box	10100001
MERSCOPE Control Box	10100002
MERSCOPE Monitor Planar	61500001
MERSCOPE Wireless Keyboard and Mouse	61500008
MERSCOPE Analysis Computer	10200001
MERSCOPE Instrument Waste Container	10300002
MERSCOPE Instrument Power Cords (regional)	Regional
MERSCOPE Instrument Connections	-
- Ethernet cable	61500004
- Fiber-optic cable	61500005
- USB cables (2x)	61500006
- Communication cable	61500007
MERSCOPE Flow Chamber	10300102
- Top	-
- Aqueduct	-
- Base	-
MERSCOPE Gasket	10300001
MERSCOPE Wash Cartridge	10700102

MERSCOPE Instrument Accessory Kit	20100005
Imaging Fluid Line Adapter (for verification experiments) (5x) <sup>§</sup>	30400010
Fluidic Line Connector (for fluidic line wash/preparation for idling) (2x) <sup>§</sup>	60900123
Immersion Oil <sup>†</sup>	30400007
Hobby Blade Handle <sup>*</sup>	30400005
Hobby Blades (pack) <sup>*</sup>	30400012
High Precision Tweezers <sup>*</sup>	30400003
Serrated Tweezers <sup>*</sup>	30400004
Lens Cleaning Tissue <sup>§</sup>	30400006
Luer-lock Syringe (5x) <sup>§</sup>	30400011

<sup>†</sup>The safety data sheet for immersion oil may be obtained from Vizgen Support ([support@vizgen.com](mailto:support@vizgen.com)).

<sup>\*</sup>For use in sample preparation. If additional parts are needed, refer to sample preparation user guides for ordering information, available online at <https://vizgen.com/>.

<sup>§</sup>The recommended supplier and part number for additional materials is provided in [Required Materials and Recommended Suppliers](#).

## IMAGING CONSUMABLES

### Vizgen Materials

Use Sample Prep Wash Buffer (PN 20300001) and Formamide Wash Buffer (PN 20300002) from the applicable MERSCOPE sample preparation kit.

MERSCOPE 140 Gene Imaging Kit, 1 sample	10400004	Storage
MERSCOPE 140 Gene Imaging Cartridge	20300017	-20°C
Imaging Buffer Activator	20300022	-20°C
DAPI and PolyT Staining Reagent	20300021	-20°C

MERSCOPE 300 Gene Imaging Kit, 1 sample	10400005	Storage
MERSCOPE 300 Gene Imaging Cartridge	20300018	-20°C
Imaging Buffer Activator	20300022	-20°C
DAPI and PolyT Staining Reagent	20300021	-20°C

MERSCOPE 500 Gene Imaging Kit, 1 sample	10400006	Storage
MERSCOPE 500 Gene Imaging Cartridge	20300019	-20°C
Imaging Buffer Activator	20300022	-20°C
DAPI and PolyT Staining Reagent	20300021	-20°C

Safety Data Sheets are available online at <https://vizgen.com/>

## Required Materials and Recommended Suppliers

Item	Vendor	Part number
<b>Buffers and additives</b>		
Ethyl Alcohol, Pure (200 proof)	Millipore-Sigma	E7023-6X500ML
RNase Inhibitor, Murine	NEB	M0314L
<b>Solutions and consumables</b>		
Fluidic Line Connector (for fluidic line wash/preparation for idling)	McMaster-Carr	7033T21
Mineral Oil	Millipore-Sigma	M5904-6X500ML
RNaseZap RNase Decontamination Solution	Thermo Fisher	AM9782
Lens Tissue	Thorlabs	MC-5
Serological Pipet (25 mL)	VWR	82051-182
Cleaning tissue (Kimwipe or similar)	VWR*	21913-214*
Imaging Fluid Line Adapter (for verification experiments)	VWR	45508-22
Luer-lock Syringe	VWR	76290-380
1000- $\mu$ L and 200- $\mu$ L pipette and compatible tips	-	-
*Alternative to Kimwipe.		

## Laboratory Setup

General laboratory equipment required (in addition to equipment used for sample preparation). General laboratory equipment should be used per manufacturer's instructions.

- Water bath (e.g., VWR 76308-896). If using an alternate make/model, it should be large enough to accommodate the MERSCOPE Imaging Cartridge: 8 × 11 in (20 × 28 cm).
- Ultrasonic water bath (for potential troubleshooting of the MERSCOPE Flow Chamber disassembly).

General laboratory equipment should be used per manufacturer's instructions.

## MERSCOPE INSTRUMENT IMAGING SUMMARY

Step	Summary
From Sample Preparation	<ul style="list-style-type: none"> <li>➔ Sample is on a MERSCOPE Slide in a solution/buffer</li> </ul>
Stain	<ul style="list-style-type: none"> <li>➔ On the bench, stain the sample on a MERSCOPE Slide with DAPI and PolyT stain</li> </ul>
<b>Configure</b>	<ul style="list-style-type: none"> <li>➔ Start MERFISH in the MERSCOPE Instrument user interface. Configure the MERSCOPE Instrument with sample details</li> <li>➔ Specify the panel-specific MERSCOPE Codebook</li> </ul>
<b>Load Cartridge</b>	<ul style="list-style-type: none"> <li>➔ Activate a thawed MERSCOPE Imaging Cartridge by manual addition of Imaging Activation Mix and a layer of mineral oil</li> <li>➔ Insert the activated imaging cartridge and prime the fluidic lines</li> </ul>
<b>Load Flow Chamber</b>	<ul style="list-style-type: none"> <li>➔ Assemble the stained MERSCOPE Slide into the MERSCOPE Flow Chamber</li> <li>➔ Connect the assembled flow chamber to the MERSCOPE Instrument fluidic lines</li> <li>➔ Wet the flow chamber and ensure there are no air bubbles</li> <li>➔ Insert the wetted flow chamber into the instrument and lock into place</li> </ul>
<b>Select</b>	<ul style="list-style-type: none"> <li>➔ Acquire a low-resolution mosaic (fully automated) and define regions of interest – up to 10 regions can be selected with a total area of up to 100 mm<sup>2</sup></li> </ul>
<b>Switch Objective</b>	<ul style="list-style-type: none"> <li>➔ Remove the assembled MERSCOPE Flow Chamber from the MERSCOPE Instrument WITHOUT detaching the fluidic lines</li> <li>➔ Apply immersion oil to the high-magnification objective</li> <li>➔ Re-insert the flow chamber into the instrument and lock into place</li> </ul>
<b>Experiment</b>	<ul style="list-style-type: none"> <li>➔ Acquire sample data (fully automated)</li> <li>➔ After the experiment is complete, select Segmentation Parameters</li> <li>➔ After segmentation parameters are selected, review the selection, and initiate image processing analysis</li> </ul>
<b>Clean</b>	<ul style="list-style-type: none"> <li>➔ Empty the waste container, remove the MERSCOPE Flow Chamber from the MERSCOPE Instrument and clean the immersion oil from the high-magnification objective. Install a Fluidic Line Connector</li> <li>➔ Disassemble and clean the MERSCOPE Flow Chamber. Leave the MERSCOPE Imaging Cartridge in place until the next sample. IF the instrument will idle or only run verification for ≥2 weeks, follow the idle period preparation procedure</li> </ul>
Transfer	<ul style="list-style-type: none"> <li>➔ Data may be transferred off the MERSCOPE Instrument from Z:\merfish_output after image processing is complete (via ethernet port or portable hard drive)</li> </ul>

## MERSCOPE INSTRUMENT IMAGING STEP-BY-STEP

Sample preparation user guides instruct users on sample preparation through readiness for imaging. Prepared samples may be stored in a sealed petri dish in Clearing Solution/Clearing Premix at 37°C for up to 7 days. Refer to the applicable sample preparation for specific instructions, available online at <https://vizgen.com/>. When ready, prepare for imaging and then proceed to DAPI and PolyT staining on the bench.

In this user guide, the term MERSCOPE Slide refers to any MERSCOPE Slide used in sample preparation.

### Prepare for Imaging

- ➔ Take Sample Prep Wash Buffer (PN 20300001) and Formamide Wash Buffer (PN 20300002) from the applicable MERSCOPE sample preparation kit.
- ➔ Prepare a 37°C water bath with ~2 cm (height) of water in the bath (or such that the water level rises to ~2.5 cm up the outside of the cartridge). Place the applicable MERSCOPE Imaging Cartridge in the 37°C water bath for 60 min. **DO NOT** allow the valve to come into contact with the water.

*Not all reagents thaw at the same rate. Incubate in the water bath for the full 60 min.*

- ➔ Warm up DAPI and PolyT Staining Reagent (PN 20300021) for 10 min in a 37°C water bath. Gently vortex the tube on the lowest setting to ensure the reagents are well mixed, and no precipitate is visible before use.
- ➔ Maintain Imaging Buffer Activator (PN 20300022) and RNase inhibitor in a benchtop cooler until use. Spin down using a benchtop centrifuge before use.
- ➔ Return unused reagents (stored at –20°C) to –20°C storage but minimize freeze-thaw cycles.
- ➔ Note the MERSCOPE Imaging Cartridge barcode number in case it must be entered manually.
- ➔ Ensure the applicable panel-specific MERSCOPE Codebook is available. It may be imported from local storage or the Vizgen Cloud.
- ➔ Ensure the MERSCOPE Instrument waste container is empty before starting an experiment.

## Prepare for Imaging after an Idle Period

- ➔ **IF** the MERSCOPE Instrument was prepared for an idle period using the [MERSCOPE Instrument Idle Procedure](#), follow the [MERSCOPE Instrument Restart After Idle Period](#) procedure to prepare the instrument for imaging.
- ➔ **IF** the MERSCOPE Instrument has been idle for  $\geq 2$  weeks (or only verification runs during that time) and was **NOT** prepared using the [MERSCOPE Instrument Idle Procedure](#), contact Vizgen Support ([support@vizgen.com](mailto:support@vizgen.com)) for advice.

## Stain the MERSCOPE Slide with DAPI and PolyT Stain

**PROTECT FROM LIGHT** at all times during staining and after the sample is mounted in the MERSCOPE Flow Chamber, until loaded in the MERSCOPE Instrument.  
Formamide Wash Buffer is hazardous. Perform these steps in a fume hood.

- ➔ Aspirate the Clearing Solution/Clearing Premix/Formamide Buffer (from sample preparation), and ensure all Clearing Solution/Clearing Premix/Formamide Buffer is aspirated from the petri dish.
- ➔ Wash **2x** with **5 mL** Sample Prep Wash Buffer.
- ➔ On the lowest setting, gently vortex the DAPI and PolyT Staining Reagent tube to ensure the reagent is well mixed and no precipitate is visible.
- ➔ Add **3 mL** DAPI and PolyT Staining Reagent, incubate 15 min on a rocker.
- ➔ Wash **1x** with **5 mL** Formamide Wash Buffer, incubate 10 min.
- ➔ Wash **1x** with **5 mL** Sample Prep Wash Buffer.
- ➔ Proceed immediately to the next step.



## MERSCOPE Instrument Operations

### Configure the Experiment – Start MERFISH

- ➔ Click **Start MERFISH** on the display and allow 1-2 min for the MERSCOPE Instrument to initialize.
- *Prior to starting a new MERFISH experiment, there must be at least 7.5 TB of disk space available on the MERSCOPE Instrument Computer and 2 TB of disk space available on the MERSCOPE Analysis Computer. If insufficient space is available, old datasets should be copied to network storage or a portable USB hard drive.*
- ➔ Enter experimental details (name, description).
- ➔ Select the applicable panel-specific MERSCOPE Codebook (may be imported from local storage or the Vizgen Cloud).
- ➔ Click **Next**.
- *Use **Next** and **Back** to navigate through the configuration screens.*
- ➔ Specify sample **Thickness**. If sample thickness is unknown, 10 µm should be selected. For FFPE samples, 10 µm should be selected regardless of the sample thickness during tissue sectioning.

The **Additional Stains** menu is used to define auxiliary bits (Aux [bit number]) based on additional staining that may have occurred during sample preparation:

- *Cell boundary staining*
- *Sequential gene encoding with the gene panel*
- *Protein staining*

- ➔ **IF** the sample was stained with the MERSCOPE Cell Boundary Stain Kit, toggle on **Cell boundary stains** under **Additional Stains**.
- *Toggling on **Cell boundary stains** automatically enables Aux 1-3.*
- ➔ **IF** the gene panel contains sequential genes, check the corresponding auxiliary bit and select **RNA** from the menu to the right of the auxiliary bit under **Additional Stains**.
- *Navigate to the panel summary page for a constructed gene panel in the MERSCOPE Gene Panel Design Software. Sequential genes are listed along with the assigned auxiliary bits.*

- ➔ **IF** the sample was stained with one or more MERSCOPE Protein Stain Kits, check the corresponding auxiliary bit(s) under **Additional Stains**.

Primary Antibody Raised In	Corresponding Protein Stain	Auxiliary Bit
Mouse	Anti-Mouse Aux 4 Protein Stain	Aux 4
Rabbit	Anti-Rabbit Aux 5 Protein Stain	Aux 5
Goat	Anti-Goat Aux 6 Protein Stain	Aux 6
Rat	Anti-Rat Aux 7 Protein Stain	Aux 7
Human	Anti-Human Aux 8 Protein Stain	Aux 8
Chicken	Anti-Chicken Aux 9 Protein Stain	Aux 9

- *Users can change the names of additional stains after they are enabled by clicking on the box.*
- ➔ Select the illumination intensity **Protein (Bright)**, **Protein (Medium)**, or **Protein (Dim)** from the menu to the right of each applicable auxiliary bit under **Additional Stains**.
- *Users should have established the illumination intensity for each channel during verification. Refer to the MERSCOPE Protein Stain Verification Kit User Guide for more information.*
- ➔ Click **Next**.
- ➔ At the end of configuration, a **Configure Summary** will appear.
- ➔ If the Configure Summary is satisfactory, click **Next**.
- *DAPI and PolyT stains are always imaged automatically.*

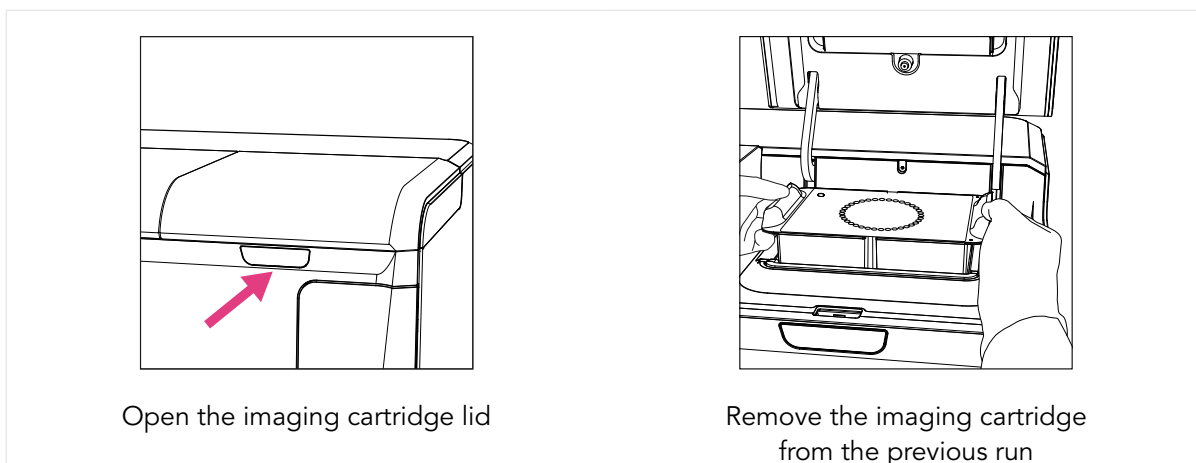
## Load – MERSCOPE Imaging Cartridge Activation and Loading

**ONLY activate** a MERSCOPE Imaging Cartridge when ready to proceed immediately with an experiment.

**DO NOT** activate a MERSCOPE Imaging Cartridge while it is thawing in the water bath.

A MERSCOPE Imaging Cartridge should be inserted into the instrument directly after activation and layering with mineral oil.

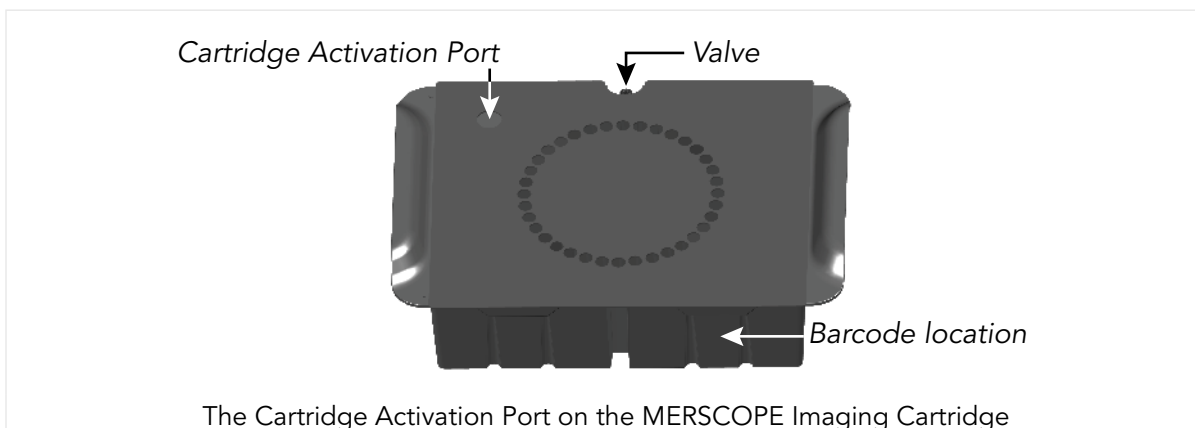
- ➔ Open the imaging cartridge lid and remove the MERSCOPE Imaging Cartridge from the previous run from the MERSCOPE Instrument. Discard the old imaging cartridge. The imaging cartridge contains hazardous materials and should be discarded per applicable institutional hazardous waste procedures. **IF** the imaging cartridge was removed as part of a maintenance/idle wash program, go directly to the next step.



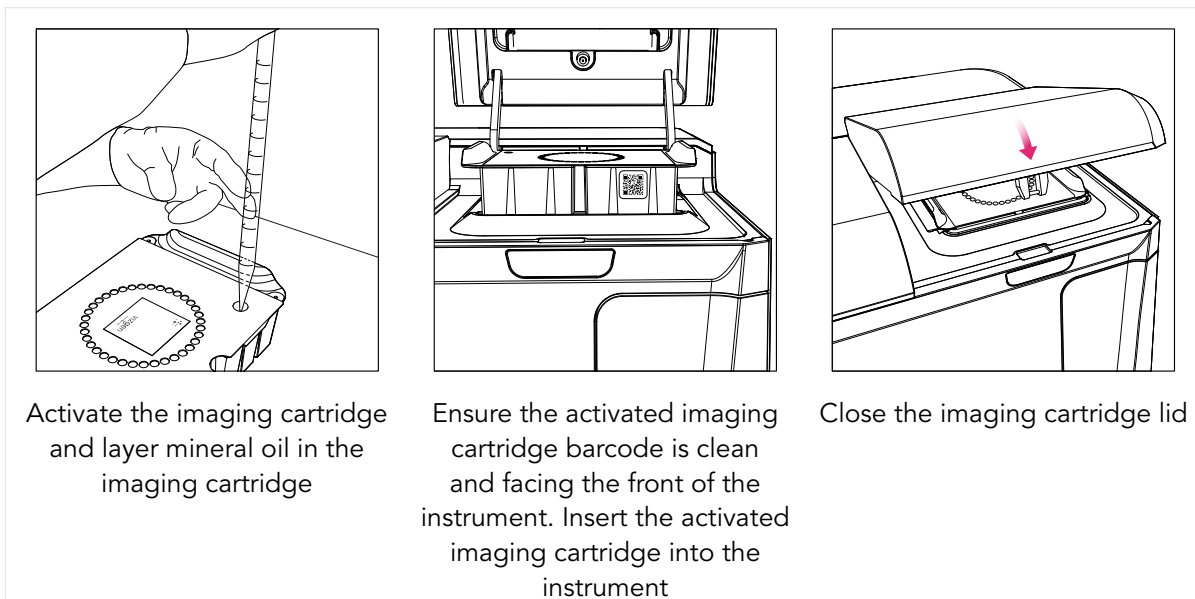
- ➔ Remove the new, thawed MERSCOPE Imaging Cartridge from the water bath and dry the outer surfaces, especially the bottom surface. Ensure the barcode is free of any liquid and note the number on the barcode.
- ➔ **Prior to** piercing the Cartridge Activation Port and cartridge activation, slowly invert the thawed MERSCOPE Imaging Cartridge **10x** to ensure the cartridge reagents are mixed.
- ➔ Clean the valve of the MERSCOPE Imaging Cartridge (refer to next page for image) by spraying RNaseZap solution onto a Kimwipe and wiping the valve, followed by spraying 70% ethanol onto a Kimwipe and wiping the valve. Users may also clean the connection in the imaging cartridge lid if contamination is a concern.
- ➔ Prepare Imaging Activation Mix:

<b>Imaging Activation Mix</b>	<b>1 sample</b>
Imaging Buffer Activator (PN 20300022)	250 µL
RNase inhibitor	100 µL

- ➔ **Prior to** inserting the MERSCOPE Imaging Cartridge into the MERSCOPE Instrument, pierce the foil at the designated Cartridge Activation Port (rear left-hand side) with a clean pipette tip. **ENSURE** the foil in the port is completely open.



- ➔ With a 1-mL pipette set to 1 mL, carefully add all the Imaging Activation Mix via the Cartridge Activation Port by inserting the pipette tip below the level of the liquid in the MERSCOPE Imaging Cartridge before dispensing. **Without changing** the pipette tip, lower and raise the pipette plunger **10x** at moderate speed to thoroughly mix the solution in the imaging cartridge, but without introducing air bubbles. **DO NOT** invert the imaging cartridge after the Cartridge Activation Port has been punctured.
- ➔ With a 25-mL serological pipette, carefully layer **15 mL** mineral oil over the liquid in the MERSCOPE Imaging Cartridge via the Cartridge Activation Port.
- ➔ Again, ensure the barcode on the MERSCOPE Imaging Cartridge is free of any liquid, and the outside/underside of the cartridge is also dry. Insert the activated imaging cartridge into the MERSCOPE Instrument with valve toward the back and the barcode toward the front.



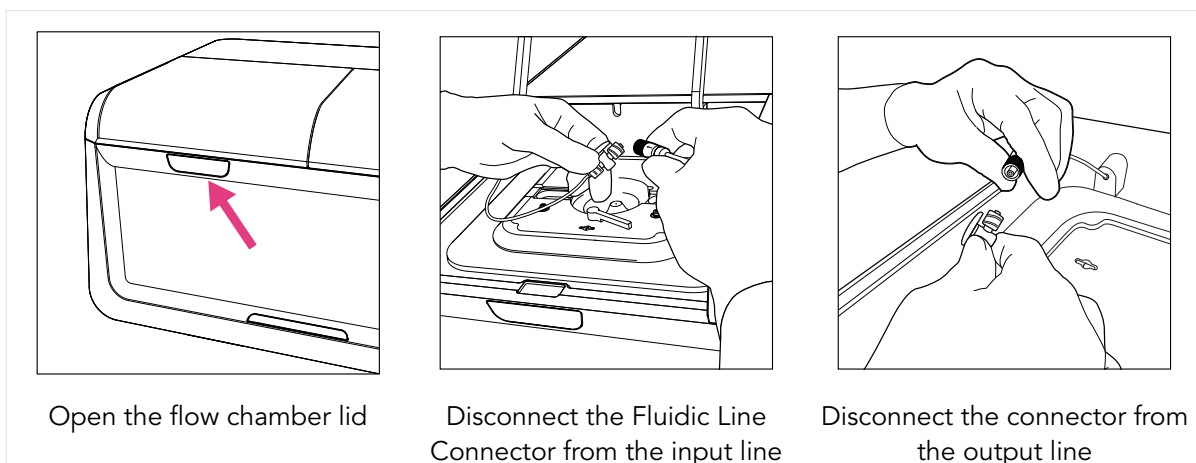
- ➔ Close the imaging cartridge lid and click **Scan Barcode**.
- *The MERSCOPE Instrument scans the MERSCOPE Imaging Cartridge barcode for compatibility with the selected MERSCOPE Codebook. If the MERSCOPE Instrument cannot read the MERSCOPE Imaging Cartridge barcode, the barcode number may be entered manually.*
- ➔ If the barcode validation is successful, click **Prime Fluidics**.
- *The MERSCOPE Instrument will proceed to prime the fluidics (this takes 2-3 min).*

## Load – MERSCOPE Flow Chamber

*In general, the MERSCOPE Flow Chamber should be lifted out of, and placed into, the stage adapter by gently holding the fluidic lines to either side of the flow chamber and lifting/placing vertically (i.e., do not tilt the Aqueduct).*

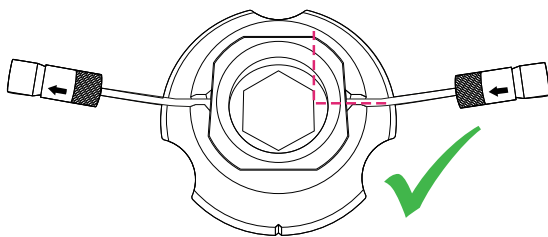
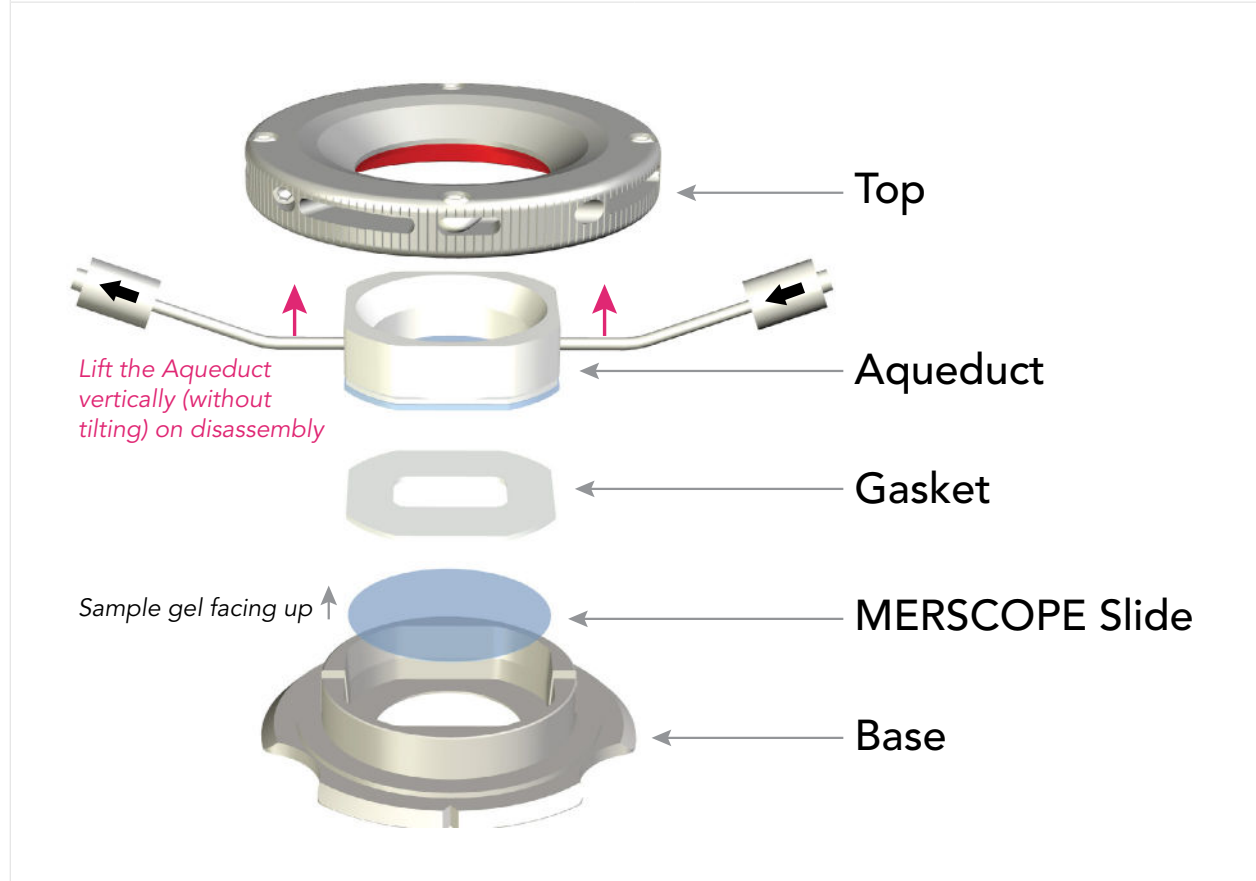
*Click **Next** to advance through screens as operations are performed.*

- ➔ Open the flow chamber lid.
- *The user interface guides users through removal of the MERSCOPE Flow Chamber in case it was not performed as part of the last Clean procedure. The instructions below assume the previous MERSCOPE Slide was disposed of in the last Clean procedure.*
- ➔ Disconnect the Fluidic Line Connector from the input line and the output line.



- ➔ Rotate the MERSCOPE Flow Chamber Top counterclockwise to disassemble the flow chamber (refer to next pages for images) and clean the MERSCOPE Gasket, Base, and Aqueduct by spraying with RNaseZap solution and wiping with a Kimwipe, followed by spraying with 70% ethanol and wiping with a Kimwipe.
- ➔ Hold the MERSCOPE Flow Chamber Base close to the sample petri dish. Gently pick up the MERSCOPE Slide with tweezers and place into the Base (sample gel facing up).
- ➔ Assemble the MERSCOPE Flow Chamber by placing the MERSCOPE Gasket on top of the MERSCOPE Slide (refer to next page for image of correct gasket orientation).
- ➔ Assemble the Aqueduct and Top. Ensure the notch in the Base and the flow direction arrows marked on the Aqueduct connectors are oriented correctly (refer to next pages for images). Twist the Top clockwise until a loud click is heard to ensure secure assembly.
- ➔ Once assembled, spray the bottom of the MERSCOPE Slide with 100% ethanol and wipe clean with a Kimwipe. Repeat **2x** more (3x total) to ensure the optical imaging surface is clean.

MERSCOPE Flow Chamber Part Names and Gasket Orientation



The MERSCOPE Gasket should be orientated with a long side of the hexagon positioned perpendicular to the fluid input line

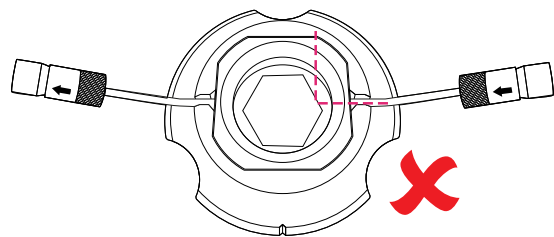
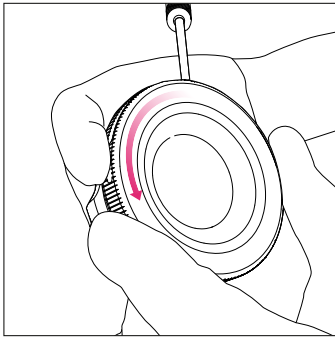
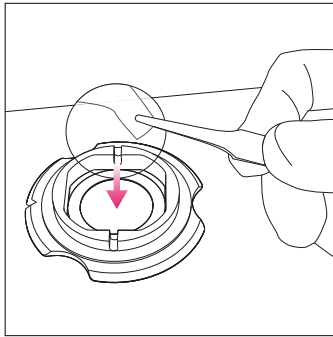


Illustration of an INCORRECT orientation of the MERSCOPE Gasket

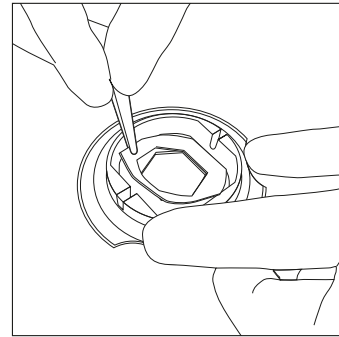
### MERSCOPE Flow Chamber Assembly and Cleaning



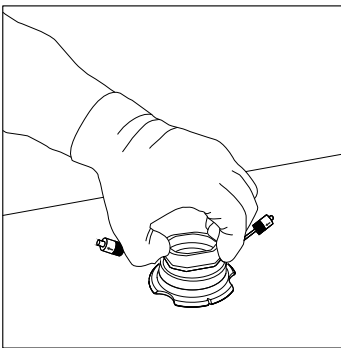
Rotate the Top counterclockwise to disassemble the flow chamber. Clean the Gasket, Base, and Aqueduct



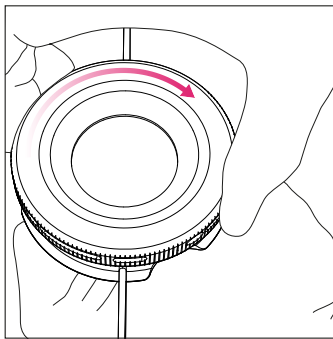
Place the new MERSCOPE Slide in the Base, sample gel facing up



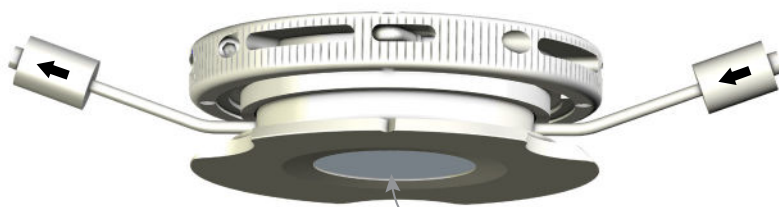
Place the MERSCOPE Gasket on top of the MERSCOPE Slide. Refer to previous page for correct gasket orientation



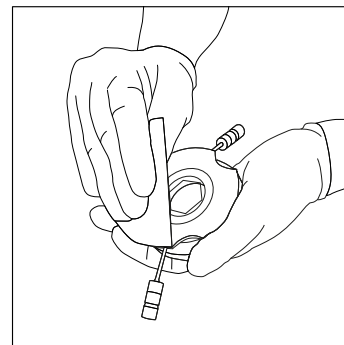
Assemble the Aqueduct. Ensure the notch in the Base and the flow direction arrows marked on the Aqueduct connectors are oriented correctly



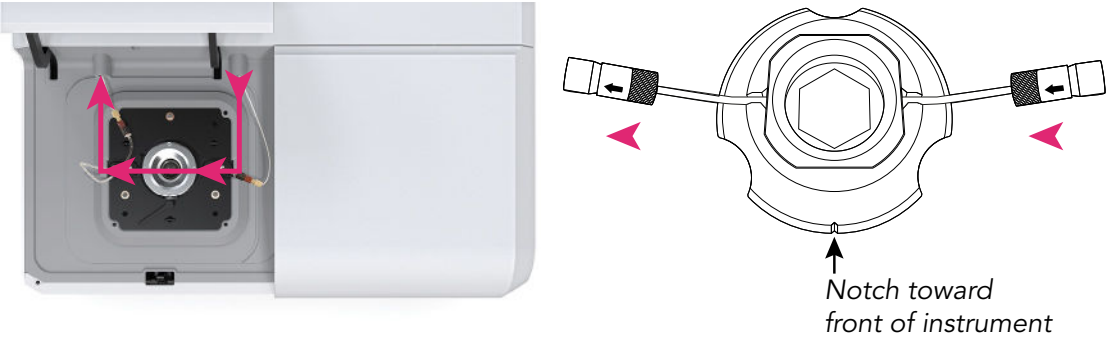
Assemble the Top and twist the Top clockwise until a loud click is heard



Clean the optical imaging surface







The MERSCOPE Instrument pulls fluid from right to left (pink arrows indicate the direction of flow).

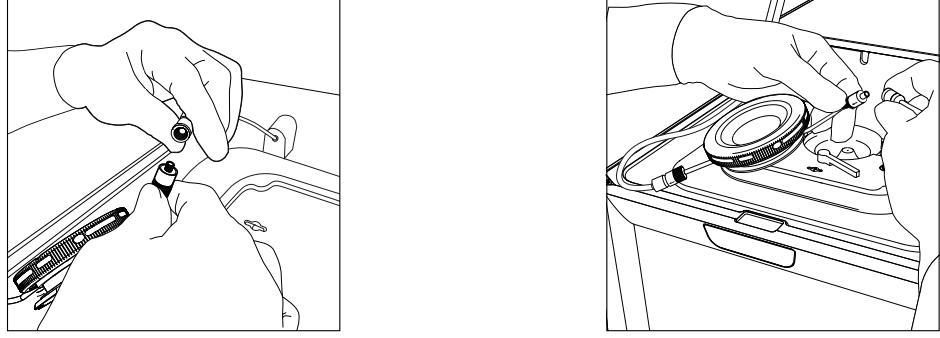
It is critical to insert the MERSCOPE Flow Chamber into the MERSCOPE Instrument in the correct orientation.

The Aqueduct has flow direction arrows marked on the connectors (illustrated without the Top in place).

The flow chamber should be connected to and inserted into the instrument with the notch in the Base toward the front of the instrument.

Notch toward front of instrument

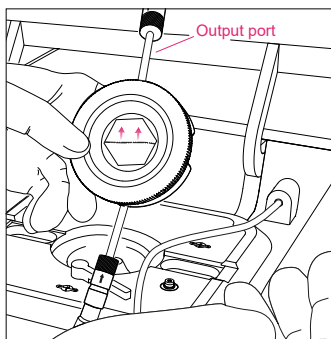
- ➔ Connect the assembled MERSCOPE Flow Chamber fluidic lines to those in the MERSCOPE Instrument. First connect the output line (to the left). Then connect the input line (to the right).



Connect the flow chamber output line to the instrument (to the left)

Connect the flow chamber input line to the instrument (to the right)

- ➔ Click **Wet Flow Chamber** to initiate wetting the MERSCOPE Flow Chamber. Orient the flow chamber vertically with the output lines upwards as flow starts to pull the air bubbles through the flow chamber and fluidic lines (refer to next page for image).



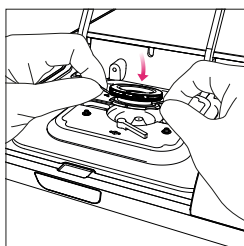
Orient the flow chamber vertically with the output lines upwards while filling

- ➔ If air bubbles are visible in the MERSCOPE Flow Chamber or input line, click **Pull more liquid**. If/when no air bubbles remain, click **Next**.

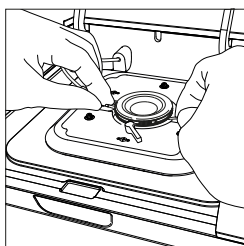
*It is only possible to **Pull more liquid** twice. If air bubbles are still visible after pulling more liquid once, ensure the fluid connections are correctly assembled and tightly closed before pulling liquid for a second time.*

*If air bubbles remain after pulling liquid for a second time, contact Vizgen Support ([support@vizgen.com](mailto:support@vizgen.com)).*

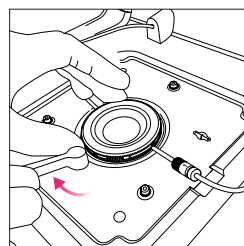
- ➔ Insert the MERSCOPE Flow Chamber into the MERSCOPE Instrument. First insert the back edge of the flow chamber under the overhangs in the stage adapter. Then, lower the flow chamber to lie flat within the stage adapter.
- ➔ Lock the MERSCOPE Flow Chamber into place.
  - *If the locking mechanism will not engage, it is possible that the MERSCOPE Flow Chamber has not been inserted correctly under the overhangs in the stage adapter. Remove the flow chamber from the stage adapter and try the insertion again, ensuring the back edge of the of the flow chamber is first inserted under the overhangs.*
- ➔ Close the flow chamber lid and click **Acquire Mosaic** to acquire a low-resolution mosaic.



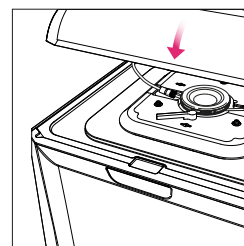
Insert the back edge of the flow chamber under the overhangs in the stage adapter



Lower the flow chamber to lie flat within the stage adapter



Lock the flow chamber into place



Close the flow chamber lid

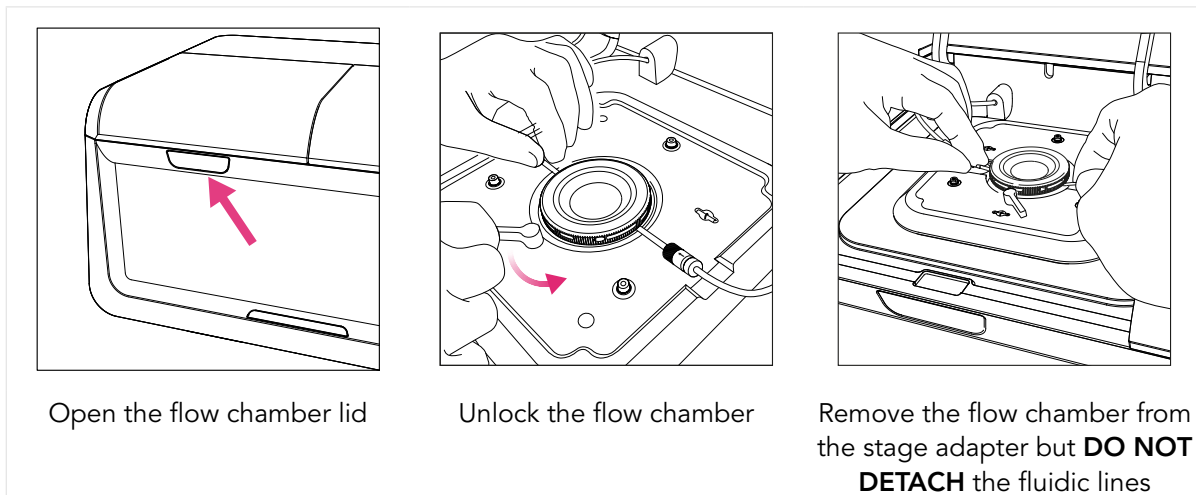
## Select Regions of Interest

*This step is done using a low-magnification objective.*

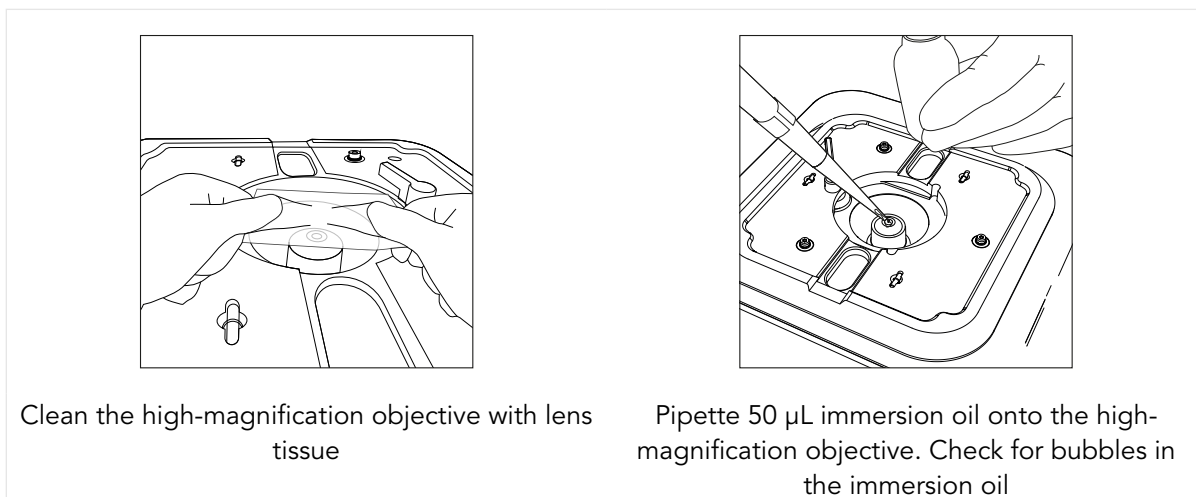
- *The MERSCOPE Instrument will acquire a low-resolution mosaic.*
- ➔ Select the regions of interest to be included in the experiment using the touchscreen or mouse. Draw a boundary on the mosaic to define the region of interest for MERFISH imaging. Once a boundary is drawn it is saved and a summary appears on the right-hand side of the screen.
- ➔ Drawing another boundary automatically creates a new region.
- ➔ Select an existing region by clicking on it on the right-hand side of the screen. When a region is selected, hold and drag a boundary dot to change its location (to redefine the boundary). Click **Done** to exit out of a selected region.
- ➔ The regions can be renamed at any time by touching the name.
- ➔ Up to 10 regions can be selected with a **total area of up to 100 mm<sup>2</sup>** (1 cm<sup>2</sup>).
- ➔ Toggle between **PolyT** and **DAPI** and move the **Visible Intensity Range** slider to adjust the contrast of the image.
- ➔ When selections are complete, click **Next**.
- *The MERSCOPE Instrument calculates the estimated time to image the selected areas. The total instrument time depends on the selected area and the number of imaging rounds.*

### Switch to the High-Magnification Objective

- ➔ Open the flow chamber lid and unlock and remove the MERSCOPE Flow Chamber from the stage adapter but **DO NOT DETACH** the fluidic lines. Click **Next** to advance through screens as operations are performed.



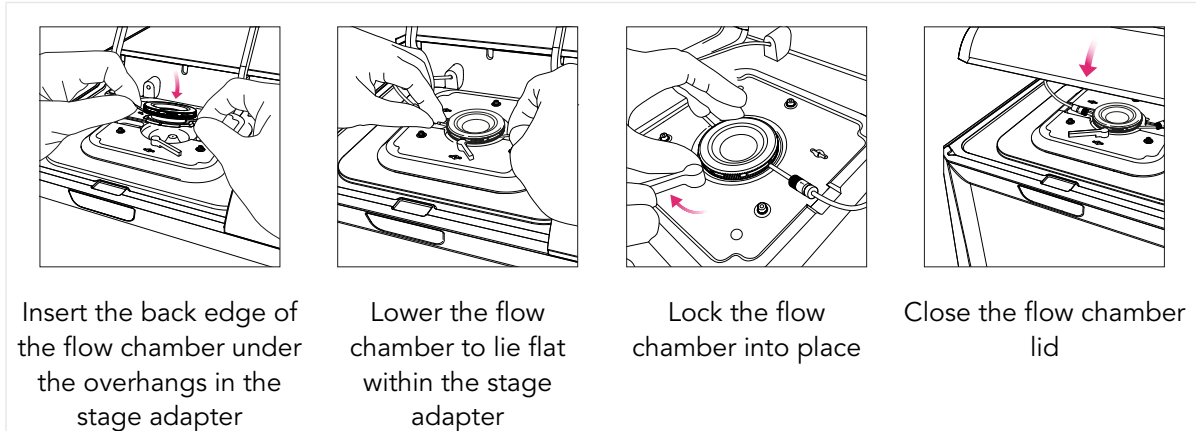
- ➔ Clean the immersion oil from the high-magnification objective with lens tissue.
- ➔ Pipette **50  $\mu$ L** fresh immersion oil onto the high-magnification objective. To ensure there are no air bubbles in the immersion oil, pipette the viscous liquid slowly and hold the pipette tip in the immersion oil for at least 20 sec. If air bubbles are present, clean off the immersion oil using lens tissue and repeat the application with fresh immersion oil.



- ➔ Re-insert the MERSCOPE Flow Chamber into the MERSCOPE Instrument. First insert the back edge of the flow chamber under the overhangs in the stage adapter. Then, lower the flow chamber to lie flat within the stage adapter.
- ➔ Lock the MERSCOPE Flow Chamber into place.

- *If the locking mechanism will not engage, it is possible that the MERSCOPE Flow Chamber has not been inserted correctly under the overhangs in the stage adapter. Remove the flow chamber from the stage adapter and try the insertion again, ensuring the back edge of the of the flow chamber is first inserted under the overhangs.*

➔ Close the flow chamber lid and click **Acquire Focus**.



## Experiment – Data Acquisition

- The MERSCOPE Instrument will attempt to find the focal plane with the high-magnification objective.
- ➔ If the focusing is successful, click **Next** to advance to an **Experiment Summary**.

If the MERSCOPE Instrument is unable to find the focal plane or detects an air bubble in the immersion oil, the user interface will instruct users to try again with immersion oil application.

- ➔ If the **Experiment Summary** is satisfactory, click **Start Measurement** to initiate the fully-automated experiment.
- The MERSCOPE Instrument will automatically run the sample. A progress bar reports progress within each imaging round.
- The MERSCOPE Instrument will indicate **Done!** when all measurements are complete.

## Segmentation Parameters and Image Processing

- ➔ After the experiment is completed, **Segmentation Parameters** can be selected. If segmentation parameters are selected, the image processing will run the selected segmentation algorithm to produce single-cell outputs. The selection of segmentation parameters can also be skipped by clicking **Skip Selection** at the bottom of the screen.
- ➔ The Segmentation Parameters screen allows users to select different fields of view to evaluate segmentation parameters in each field of view. Segmentation options:
  - Watershed segmentation identifies individual cells by approximating cell space starting from a central starting point (Seed Stain) and ending at an approximated cell boundary based on a second stain (Watershed Stain). Watershed segmentation may be applied based on Seed Stain (e.g., DAPI) and Watershed Stain (e.g., PolyT).
  - Cellpose segmentation identifies individual cells by approximating cell boundaries from detected stains and filling cell space from these boundaries. Cellpose segmentation (1, 2, 3) is available if cell boundary stains were used in the measurement.
    - Cellpose: a generalist algorithm for cellular segmentation, Nat Methods 18, 100-106 (2021). <https://doi.org/10.1038/s41592-020-01018-x>
- ➔ Select one of the two options for segmentation including the seed stain/watershed stain or boundary stain.
- ➔ Segmentation results will be displayed on the image below. On the left side, the image channel displayed may be selected and the Visible Intensity Range slider may be adjusted to facilitate evaluation of the segmentation results.

- *After segmentation parameters are defined (or skipped), an Image Processing Parameters screen will appear.*
- ➔ If the **Image Processing Parameters** are satisfactory, click **Start Image Processing** to initiate image processing analysis.
- *The MERSCOPE Instrument will automatically copy the raw image data to the MERSCOPE Analysis Computer to begin or queue the image processing analysis.*
- *Image processing analysis will continue in the background after an experiment is completed on the MERSCOPE Instrument.*
- ➔ Click **Clean Instrument** to proceed to cleaning the MERSCOPE Instrument. The Clean process and a new experiment can begin on the instrument while image processing analysis of previous experiments is running in the background.
- ➔ Once image processing analysis is complete, the results will be available at Z:\merfish\_ output and should be copied off the MERSCOPE Instrument either through the 10 Gb ethernet port or through a portable hard drive plugged into the USB-C port on the front of the instrument.

## Clean

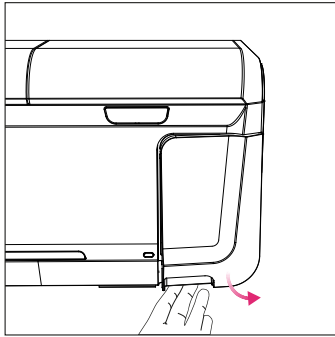
**IF** the MERSCOPE Instrument will not be used to run a MERFISH experiment for  $\geq 2$  weeks, prepare the instrument per the [MERSCOPE Instrument Idle Procedure](#) **IN ADDITION TO (AFTER)** the Clean procedure.

**IF** verification is the only experiment planned for  $\geq 2$  weeks, also use the [MERSCOPE Instrument Idle Procedure](#) (as verification runs do not use fluidics).

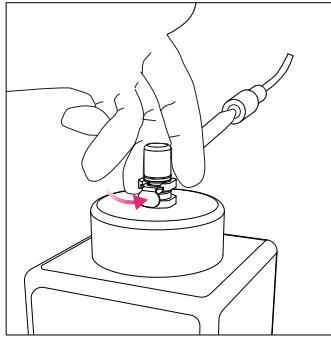
- ➔ The MERSCOPE Instrument executes automatic fluidic line washes. The user interface also guides users through (1) emptying the waste container, (2) removing the MERSCOPE Flow Chamber and cleaning the immersion oil off the high-magnification objective with lens tissue, and (3) installing a Fluidic Line Connector (PN 60900123) after an experiment is complete. Click **Next** to advance through screens as operations are performed. Refer to next page for images.
- ➔ After the MERSCOPE Flow Chamber is removed from the MERSCOPE Instrument, rotate the flow chamber Top counterclockwise to disassemble the flow chamber. Lift the Aqueduct vertically (i.e., do not tilt during removal). Discard the MERSCOPE Slide per applicable institutional hazardous waste procedures. Prepare the flow chamber for the next run ([Load – MERSCOPE Flow Chamber](#)) or for storage ([MERSCOPE Flow Chamber Idle Procedure](#)).
- ➔ Discard the waste container contents per applicable institutional hazardous waste procedures.
  - *The waste container must be below 250 g for the instrument to proceed with the next run.*
- ➔ At the end of cleaning, click **Done** to return to the home page and start the next experiment.



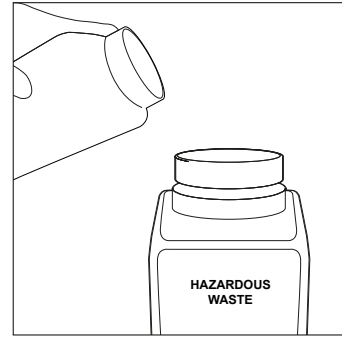
**Clean Instrument Step 1 of 3 – Empty waste container**



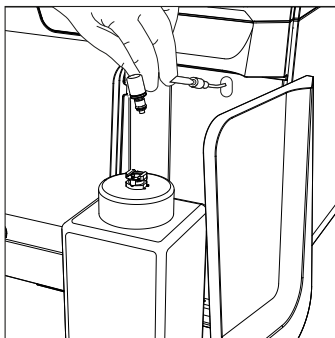
Open the waste container door



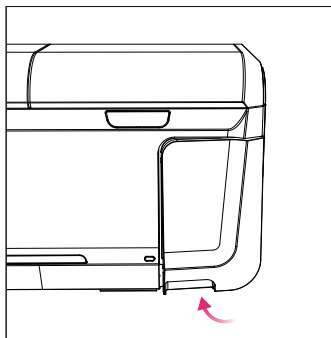
Disconnect the waste container tube



Empty the waste container

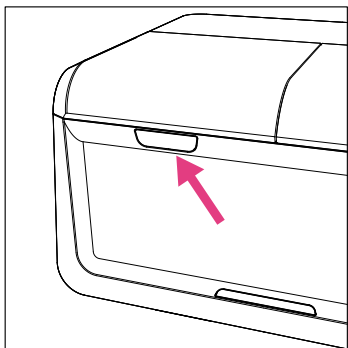


Reconnect the waste container

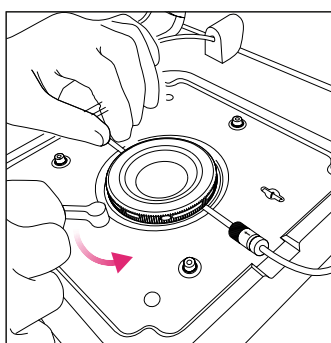


Close the waste container door

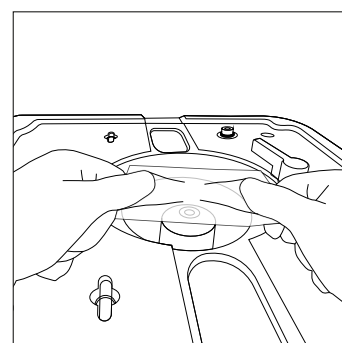
**Clean Instrument Step 2 of 3 – Remove oil from the high-magnification objective lens**



Open the flow chamber lid

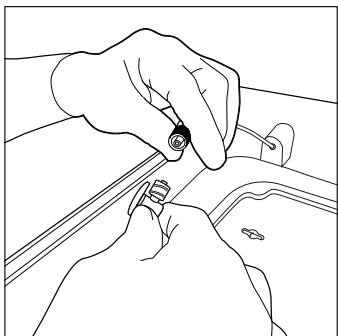


Unlock the flow chamber. Remove the flow chamber from the stage adapter and disassemble

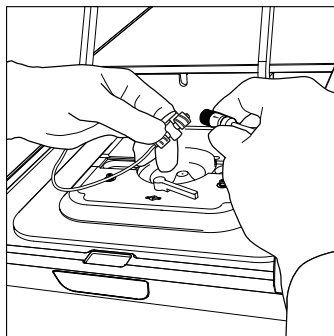


Clean the high-magnification objective with lens tissue

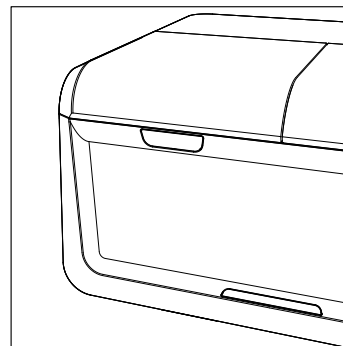
**Clean Instrument Step 3 of 3 – Connect the fluidic lines**



Connect a Fluidic Line Connector to the output line



Connect the connector to the input line to complete the circuit  
Ensure the tap of the connector is in the open orientation



Close the flow chamber lid

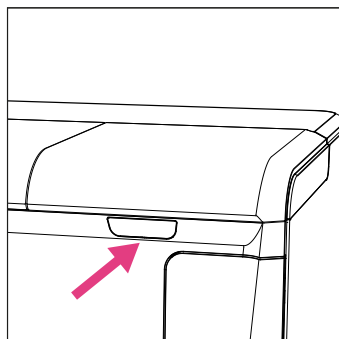
**Home Page Top Navigation Bar**

- ➔ **Experiments.** Users can browse prior experiments.
- ➔ **Maintenance.** Wash the MERSCOPE Instrument fluidic lines.
- ➔ **Help.** Users can see this document.
- ➔ **Account.** Users must log into their account to have access to prior experiments and MERSCOPE Codebooks via the Vizgen Cloud.

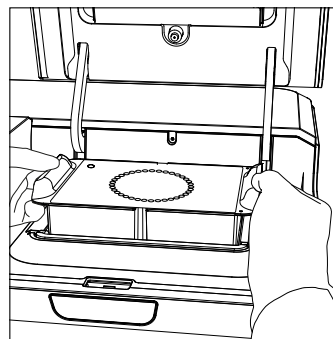
## INSTRUMENT WASH (MAINTENANCE)

Users may wash the MERSCOPE Instrument fluidic lines by implementing the wash program under **Maintenance** on the home page top navigation bar. The wash program may also be performed as part of [INSTRUMENT SHUTDOWN \(IDLE\) AND RESTART](#).

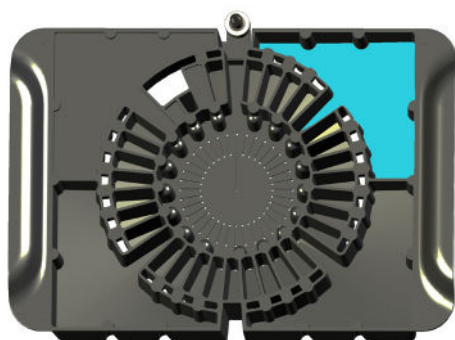
- ➔ Click **Start instrument wash** and click **Next** to advance through screens as operations are performed.
- ➔ Open the imaging cartridge lid and remove the MERSCOPE Imaging Cartridge from the previous run from the MERSCOPE Instrument. The imaging cartridge contains hazardous materials and should be discarded per applicable institutional hazardous waste procedures.
  - *The user interface guides users through removal of the MERSCOPE Flow Chamber, cleaning the high-magnification objective, and installation of Fluidic Line Connector in case it was not performed as part of the last Clean procedure.*
- ➔ Open the flow chamber lid and ensure the tap of the Fluidic Line Connector is in the open orientation. Close the flow chamber lid.
- ➔ Rinse the back right well of a MERSCOPE Wash Cartridge (PN 10700102) with nuclease-free water, where the valve indicates the back of the wash cartridge (refer to next page for image). Then, fill the back right well of the wash cartridge with **30 mL** nuclease-free water.
- ➔ Insert the filled MERSCOPE Wash Cartridge into the MERSCOPE Instrument and close the imaging cartridge lid.



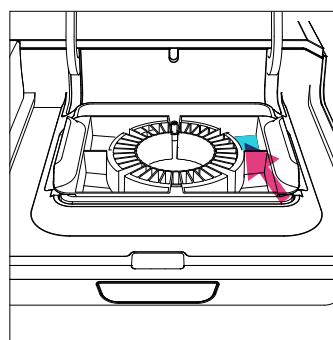
Open the imaging cartridge lid



Remove the imaging cartridge from the previous run



Rinse the back right well of a MERSCOPE Wash Cartridge with nuclease-free water. Then, fill the back right well with 30 mL nuclease-free water, where the valve indicates the back of the cartridge



Insert the wash cartridge with 30 mL nuclease-free water into the instrument and close the imaging cartridge lid

- ➔ Click **Start Fluidics Line Wash** to initialize the instrument wash.
- *A progress bar reports progress.*
- ➔ Click **Done – Empty Waste Container** when the instrument wash is complete.
- ➔ Empty the waste container per the instructions in the [Clean](#) section.
- ➔ Click **Done – Go to Home Page** to return to the home page.

When all maintenance washes are complete:

- ➔ Leave the Fluidic Line Connector in place until the next run.
- ➔ Remove the MERSCOPE Wash Cartridge from the MERSCOPE Instrument and empty the residual water into applicable institutional hazardous waste procedures. Store the wash cartridge upside down on a Kimwipe.
- ➔ Ensure both the flow chamber lid and imaging cartridge lid are closed.

## INSTRUMENT INSTALL AND OPERATION

### Initial Install and Startup

- ➔ Initial MERSCOPE Instrument install and startup is performed by an authorized Vizgen representative. Refer to the *MERSCOPE Instrument Site Preparation Guide* for more information, available online at <https://vizgen.com/>.

### Normal Day-to-Day Operations

- ➔ After an experiment is complete, the user interface returns to the home page and users can proceed with the next experiment, even while image processing analysis is running on the previous experiment.

## INSTRUMENT SHUTDOWN (IDLE) AND RESTART

### MERSCOPE Instrument Idle Procedure

*IF the MERSCOPE Instrument will not be used to run a MERFISH experiment for  $\geq 2$  weeks, prepare the instrument as outlined below.*

*IF verification is the only experiment planned for  $\geq 2$  weeks, also use this procedure (as verification runs do not use fluidics).*

- ➔ Perform the cleaning process per normal operations after the final experiment (refer to [Clean](#) or equivalent sections in verification user guides).
- ➔ Prepare the MERSCOPE Flow Chamber per the [MERSCOPE Flow Chamber Idle Procedure](#).
- ➔ Access the wash program under **Maintenance** on the home page top navigation bar.
- ➔ Follow the instructions in the [INSTRUMENT WASH \(MAINTENANCE\)](#) procedure to complete a wash program.
- ➔ Repeat the wash program **2x** more (**3x** total).
- ➔ During the third program, determine if the flow rate is acceptable per the [MERSCOPE Instrument Flow Rate Check](#).
- ➔ Finalize the [INSTRUMENT WASH \(MAINTENANCE\)](#) procedure: leave the Fluidic Line Connector in place, remove and store the MERSCOPE Wash Cartridge, and close the flow chamber lid and imaging cartridge lid.
- ➔ As applicable, shut down the MERSCOPE Instrument Computer and power down the MERSCOPE Control Box (this will also shut down the MERSCOPE Imaging Box).
  - **DO NOT** shut down the MERSCOPE Analysis Computer until all image processing analysis is complete.

### MERSCOPE Flow Chamber Idle Procedure

- ➔ After the MERSCOPE Flow Chamber is removed from the MERSCOPE Instrument, rotate the MERSCOPE Flow Chamber Top counterclockwise to disassemble the flow chamber. Lift the Aqueduct vertically (i.e., do not tilt during removal). Discard the MERSCOPE Slide per applicable institutional hazardous waste procedures.
- ➔ Clean the MERSCOPE Gasket, Base, and Aqueduct by spraying with 70% ethanol and wiping with a Kimwipe.
- ➔ Assemble the MERSCOPE Flow Chamber Base, Gasket, Aqueduct, and Top **WITHOUT** a MERSCOPE Slide in place.
- ➔ Twist the Top clockwise until a loud click is heard to ensure secure assembly.
- ➔ Store the assembled MERSCOPE Flow Chamber in a cool, dry, dark place.

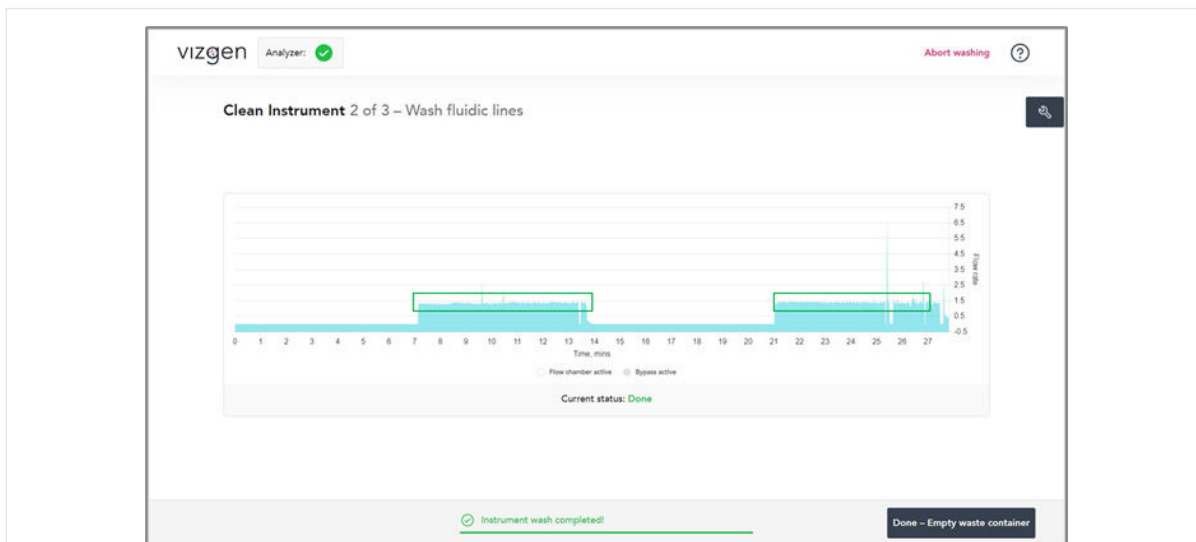
### MERSCOPE Instrument Restart After Idle Period

- ➔ As applicable, switch on the MERSCOPE Control Box (this will also start up the MERSCOPE Instrument Computer).
  - Ensure the MERSCOPE Analysis Computer is switched on.
- ➔ Follow the instructions in the [INSTRUMENT WASH \(MAINTENANCE\)](#) procedure to complete a wash program.
- ➔ Repeat the wash program **2x** more (**3x** total).
- ➔ During the third program, determine if the flow rate is acceptable per the [MERSCOPE Instrument Flow Rate Check](#).

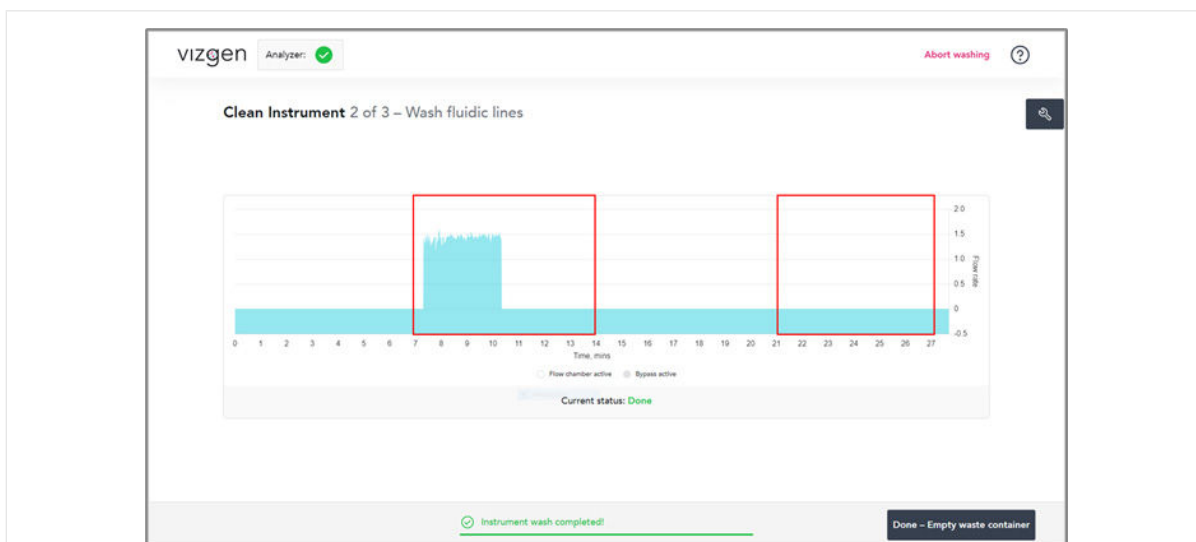
### MERSCOPE Instrument Flow Rate Check

➔ During the third program of an **INSTRUMENT WASH (MAINTENANCE)** procedure, determine if the flow rate is acceptable by observing the flow rate during minutes 7-14 and 21-27.

- The flow rate is acceptable if there are no periods of  $\geq 1$  min in which the flow rate is out of the range of 1.0 to 2.0. Proceed with an experiment per normal operations.
- The flow rate is **NOT** acceptable if the flow rate is out of the range of 1.0 to 2.0 for  $\geq 1$  min. Contact Vizgen Support ([support@vizgen.com](mailto:support@vizgen.com)) if this occurs.



**Acceptable** flow rate example. During minutes 7-14 and 21-27, there are no periods of  $\geq 1$  min where the flow rate is out of the range of 1.0 to 2.0.



**Unacceptable** flow rate example. During minutes 7-14 and 21-27, there are multiple periods of  $\geq 1$  min where the flow rate is out of the range of 1.0 to 2.0.



### MERSCOPE Instrument Restart After Extended Shutdown

- ➔ In the case that the MERSCOPE Instrument has not been used for >3 months, contact Vizgen Support ([support@vizgen.com](mailto:support@vizgen.com)) for advice on instrument startup operations.

## MERSCOPE INSTRUMENT OUTPUT FILE STRUCTURE AND FORMATS

One of the primary outputs of a MERFISH experiment is a list of the spatial locations of RNA molecules in the sample. The RNA molecules need to be pooled together into one or many gene expression profile(s) that describe biological entities. The MERSCOPE Instrument Software generates single-cell gene expression profiles. To facilitate downstream analysis of MERSCOPE gene expression data, the analysis software provides organized experimental data in open formats (JSON, PNG, CSV, TIFF, PARQUET) and a MERSCOPE Vizualizer file (VZG) that can be used to explore these outputs in a single interface.

### Analysis Output Folder Content

Analysis results of each MERFISH experiment are in a subfolder of Z:\merfish\_output\ with the same name as the experiment. In each Z:\merfish\_output\{experiment\_name}\ folder are:

- experiment.json
- PNG images
- region\_{region\_index} folders for each individual region:
  - [Region Output Folder Content – Always Available](#)
  - [Region Output Folder Content – Segmentation Dependent](#)
  - [Region Output Folder Content – Images](#)

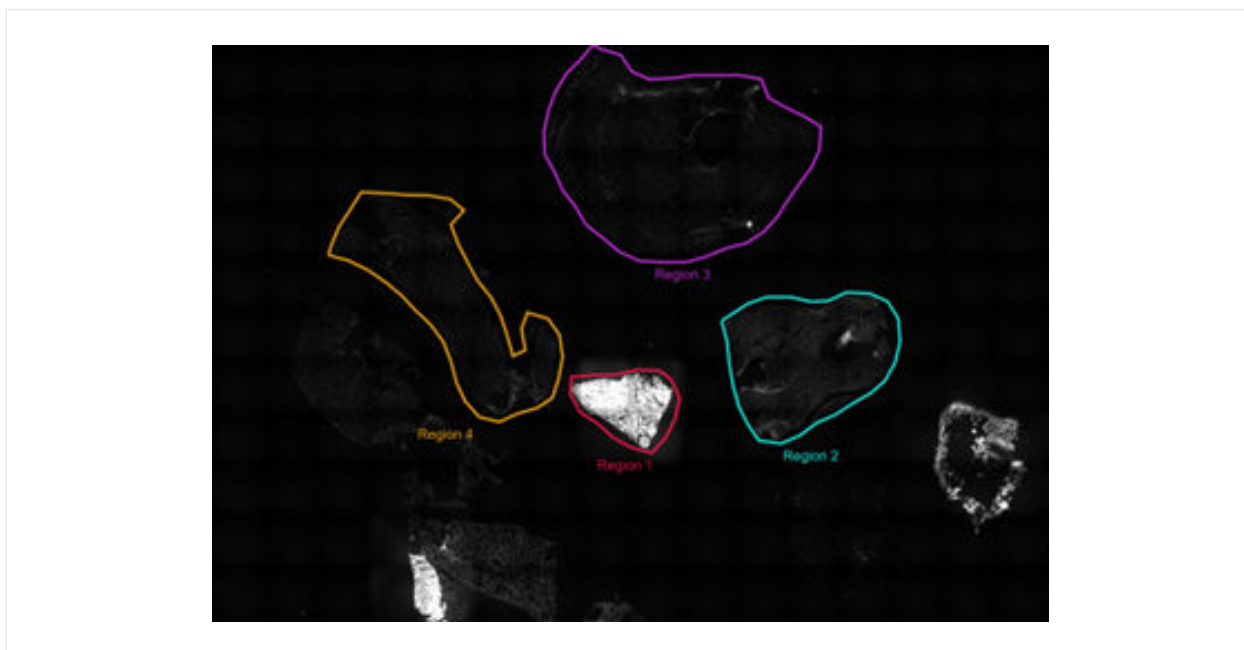
The **experiment.json** file contains metadata about the experiment: how data were collected and a summary of the analysis outputs. The top-level keys are:

- |                         |                            |
|-------------------------|----------------------------|
| • startDateTime         | • sampleThickness          |
| • userId                | • additionalStains         |
| • experimentId          | • fiducialColor            |
| • experimentName        | • usedReadouts             |
| • experimentDescription | • usedHybridizationBuffers |
| • experimentDirectory   | • cartridgeBarcode         |
| • codebookName          | • regionSummaries          |
| • codebookId            | • measurementStartDateTime |
| • panelName             | • endDateTime              |
|                         | • fovCount                 |
|                         | • runMode                  |
|                         | • cell_metrics             |

If segmentation was selected as part of analysis ([Segmentation Parameters and Image Processing](#)), the **experiment.json** file contains a per-region summary of the segmentation results and single-cell gene expression, which is saved in the cell\_metrics key. cell\_metrics contains a list with one object per region. Within each list item there two keys: region\_id, which is the index of the region (see below), and metrics, which contains the following metrics:

num_cells	The number of segmented cells in this region.
volume_stats	The distribution of cell volumes ( $\mu\text{m}^3$ ).
transcript_count_stats	The distribution of transcripts per cell (count).
ratio_covered	The fraction of the total experimental space covered by a cell.
ratio_transcripts_within_cell	The fraction of all transcripts within a cell.
num_cells_with_no_transcripts	The number of segmented cells that do not contain any transcripts.
enrichment	The per-gene fraction of transcripts within a cell.
sum_signals_medians	The per-channel median of image intensity within a cell, for all imaging channels that are stitched into a mosaic tiff image ( <a href="#">Region Output Folder Content – Images</a> ). This value is calculated for both the raw image intensity and the high-pass filtered intensity.

**PNG images** are derived from low-magnification objective imaging at the beginning of the experiment and named according to the dye. For example, dapi.png and polyt.png for DAPI and PolyT, respectively. The location of each region imaged with the high-magnification objective and analyzed as part of the experiment are overlaid on the PNG images.



Each **region\_{region\_index}** folder contains all the independently analyzed outputs for each individual region selected for imaging (Select Regions of Interest). Users may have defined unique names for individual regions during experiment setup (Select Regions of Interest). The relationship between each region index and the name of the region is saved in the **experiment.json** file under the regionSummaries key. In the example above, the region names in the low magnification mosaic above correspond to the regionSummaries below:

```
"regionSummaries": [
  {
    "name": "Region 4",
    "startIndex": 0,
    "endIndex": 299
  },
  {
    "name": "Region 1",
    "startIndex": 300,
    "endIndex": 384
  },
  {
    "name": "Region 2",
    "startIndex": 385,
    "endIndex": 645
  },
  {
    "name": "Region 3",
    "startIndex": 646,
    "endIndex": 1199
  }
]
```

In this example, the data for the regions would be saved as follows:

Region Name	Region Index	Output Subfolder
Region 4	0	region_0
Region 1	1	region_1
Region 2	2	region_2
Region 3	3	region_3

## Region Output Folder Content – Standard

In every region output folder are:

- {experiment\_name}\_region\_{region\_index}.vzg
- detected\_transcripts.csv
- summary.png

The {**experiment\_name**}\_region\_{**region\_index**}.vzg file can be opened with the MERSCOPE Vizualizer (Desktop Version). It contains all the information needed to visualize the transcript locations, cell boundaries, and a compressed version of the mosaic image channels (e.g., DAPI, PolyT, Cellbound stains, other additional stains). Refer to the *MERSCOPE Vizualizer User Guide (Desktop Version)* for more information, available online at <https://vizgen.com/>.

The **detected\_transcripts.csv** file is a standard comma separated values (CSV) formatted text file. Column headers:

[BLANK]	A numeric index that uniquely identifies a transcript within a field of view. The index is non-consecutive and ascending within each field of view.
barcode_id	The row index of the identified transcript “barcode” in the codebook file (zero indexed). fov and barcode_id are a composite primary key for the detected_transcripts.csv table.
global_x	The x-coordinate of the transcript ( $\mu\text{m}$ ), relative to the space of the experimental region. global_x may be negative in some circumstances depending on the alignment between fields of view.
global_y	The y-coordinate of the transcript ( $\mu\text{m}$ ), relative to the space of the experimental region. global_y may be negative in some circumstances depending on the alignment between fields of view.
global_z	The index of the z-position. The position is a zero-indexed integer. global_z can be translated into microns using the entry in the first row of the zPos column of the dataorganization.csv file sorted in ascending order.
x	The x-coordinate of the transcript ( $\mu\text{m}$ ), within the coordinate space of the field of view in which it was imaged.
y	The y-coordinate of the transcript ( $\mu\text{m}$ ), within the coordinate space of the field of view in which it was imaged.
fov	The index of the field of view in which the transcript was imaged (zero indexed). fov and barcode_id are a composite primary key for the detected_transcripts.csv table.
gene	The human readable name of the gene this transcript is associated with. Gene is derived from the “name” column of the codebook file.
transcript_id	A unique identifier of the gene that this transcript is associated with. transcript_id is derived from the “id” column of the codebook file.
cell_id	<b>IF</b> cell segmentation was performed: The numeric index of the cell that contains this transcript, if any. If this transcript is not associated with a cell, cell_id will be -1. cell_id maps to the EntityID field found in the cell_boundaries.parquet and cell_metadata.csv.

The **summary.png** file is a visual summary of the analysis:

- The spatial distribution of transcripts both within the region and within the mean field of view.
- The “scale factor” used to normalize the brightness of each MERFISH bit before decoding.
- The abundance versus misidentification rate of MERFISH decoding for the experiment.
- Statistics about segmentation and example images of cell boundaries if segmentation was performed.

### Region Output Folder Content – Segmentation Dependent

If segmentation was selected as part of analysis ([Segmentation Parameters and Image Processing](#)), files capturing the segmentation include:

- cell\_boundaries.parquet (MERSCOPE Instrument Software v232 or later)
- cell\_by\_gene.csv
- cell\_metadata.csv

The **cell\_boundaries.parquet** (MERSCOPE Instrument Software v232 or later) is in an open-source file format for tabular data that has efficient I/O performance and small file size compared with csv/text files (<https://parquet.apache.org/>). The **cell\_boundaries.parquet** file contains the boundaries of cells in microns, formatted as a data table using GeoPandas. This file supersedes the HDF5 formatted cell boundary files produced by earlier versions of the MERSCOPE Instrument Software. Columns:

[BLANK]	A numeric index. It is sorted and unique, but entries are not consecutive.
ID	A numeric row index that begins from zero. ID is unique within an analysis region.
EntityID	An integer (int64) identifier for a cell or other biological entity identified through spatial analysis. EntityID has the format: analysis timestamp, task index, tile index, geometry index. EntityID is guaranteed to be unique to a biological entity (e.g., cell) within an analysis region. The use of the analysis timestamp in the ID makes the EntityID likely to be unique across all experiments run on a MERSCOPE Instrument. Filtering by EntityID will enable users to get all ZLevels of a given biological entity (e.g., cell).
Name	A free-text description of the geometry in the row.
Type	The type of the entity referred to in EntityID.
ZIndex	The z-index of this slice of the biological entity in the 3D stack, corresponds to global_z in detected_transcripts.csv.
ZLevel	The z-position of this slice of the biological entity in the 3D stack ( $\mu\text{m}$ ).
Geometry	A valid WKT-format MultiPolygon that describes the biological entity (e.g., cell) at the given ZLevel. The vertices of the MultiPolygon are in microns relative to the space of the experimental region. Geometry uses MultiPolygon objects to describe biologicals entities that may be contiguous in 3D space, but discontinuous at a given ZLevel (i.e., a U-shaped cell). Even if the cell region is contiguous and can be described with a single Polygon, it is stored as a MultiPolygon for data-type consistency.
ParentID	If this biological entity (e.g., cell) is related to a higher-level ("parent") entity, the EntityID of the parent may be stored here. If this biological entity (e.g., cell) does not descend from a parent entity, this value is None.
ParentType	The type of the parent entity, if any. If there is no parent entity, the value is None.

The **cell\_by\_gene.csv** file is a standard CSV formatted text file. The first column of the file has the header “cell” and is a list of EntityID values corresponding to those in the cell\_boundaries.parquet and cell\_metadata.csv files. The remaining columns are gene names that correspond with the gene column of the detected\_transcripts.csv and the name column of the codebook.

- **IF** the cell\_by\_gene.csv file is generated by the MERSCOPE Instrument Software, it contains all the same genes as the codebook in the same order, even if no transcripts of a given gene are detected.
- **IF** the cell\_by\_gene.csv is generated by a post-processing tool that does not have access to the codebook, the number and order of genes in the file may vary.



The **cell\_metadata.csv** file is a standard CSV formatted text file. Working with the raw geometry information for an entire experiment can require significant time and memory resources. Cell metadata is calculated and provided to accelerate some types of geometric operations, such as cell filtering. Entity metadata files have the following columns:

EntityID	These IDs correspond with those in the cell_boundaries.parquet and cell_by_gene.csv files.
fov	The field of view index of the cell or None if the fov information is not available.
volume	The approximate volume of the cell ( $\mu\text{m}^3$ ). Based on a linear interpolation of each ZLevel of the cell geometries to produce a 3D solid.
center_x	The x-position of the center of the cell in the global coordinate system ( $\mu\text{m}$ ).
center_y	The y-position of the center of the cell in the global coordinate system ( $\mu\text{m}$ ).
min_x	The minimum x-extent of the cell (considering all ZLevels) in the global coordinate system ( $\mu\text{m}$ ).
max_x	The maximum x-extent of the cell (considering all ZLevels) in the global coordinate system ( $\mu\text{m}$ ).
min_y	The minimum y-extent of the cell (considering all ZLevels) in the global coordinate system ( $\mu\text{m}$ ).
max_y	The maximum y-extent of the cell (considering all ZLevels) in the global coordinate system ( $\mu\text{m}$ ).
anisotropy	The ratio of the length of the major axis of the cell to the length of its minor axis (always greater than or equal to 1). A value of 1 represents a circular or square cell.
transcript_count	The number of transcripts, including Blanks, that fall within the cell.
perimeter_area_ratio	The ratio of the perimeter of the cell to its area, calculated at each ZLevel and averaged across occupied ZLevels. Higher values correspond with more complex / non-convex shapes.
solidity	The ratio of the area of the cell to the area of a convex hull around occupied ZLevels, calculated at each ZLevel and averaged across the cell. Lower values correspond with more complex / non-convex shapes.

Furthermore, additional columns are appended for all imaging channels stitched into a mosaic tiff image ([Region Output Folder Content – Images](#)). For every cell, the total intensity (for all occupied ZLevels) of each channel within the cell boundary is calculated on the raw fluorescent image and on a high-pass filtered version of the image. These will appear as columns:

- {stain name}\_raw
- {stain name}\_high\_pass

The number of appended columns and naming depends on the configuration of each experiment.

Stain metrics can be helpful for filtering and sorting cells within an experiment. It is not recommended to compare stain metrics between experiments. Many experimental factors, including tissue clarity and image post-processing settings, may affect the values.

## Region Output Folder Content – Images

The **images** subfolder contains images of the experimental region suitable for quantitative analysis and image metadata:

- tiff images
- micron\_to\_mosaic\_pixel\_transform.csv
- manifest.json

Every image channel acquired during a MERFISH experiment that is not decoded as a MERFISH bit will be outputted as a **mosaic tiff image**. This includes DAPI, PolyT, Cellbound stains (if applicable), and subsequent round stains (if applicable). Raw data images from the MERFISH experiment are stitched together based on the alignment of fiducial beads to create a mosaic that minimizes the appearance of seams between fields of view. The images are single channel, single plane, 16-bit grayscale tiff files, with the naming convention mosaic\_{stain name}\_z{ZIndex}.tif

The **micron\_to\_mosaic\_pixel\_transform.csv** file is a space-delimited text file with no headers. To overlay transcript locations or cell geometries on the mosaic images, it is necessary to convert coordinates from microns to pixels. The contents of the file are 3 rows of 3 floating point numbers that may be read as a 3 x 3 matrix. This data is an affine transformation matrix describing translation and scaling from microns to pixels. The contents of the file have the form:

Scaling x	0	Translation x
0	Scaling y	Translation y
0	0	1

The x and y translations may be either positive or negative. The origin (0,0) of the pixel coordinate system is not necessarily in the same location as the origin of the micron coordinate system.

The **manifest.json** file contains metadata about the assembly of the mosaic images. The manifest.json file is not necessary for typical downstream analysis but is necessary for rebuilding some analysis outputs.

## TECHNICAL TIPS

### Experimental Planning

The MERSCOPE Instrument analyzes one sample at a time and imaging takes 0.5 – 1.5 days.

Sample preparation can be performed in batches and samples can be stored in Clearing Solution/Clearing Premix at 37°C for up to 7 days. Refer to the applicable sample preparation user guide for more information, available online at <https://vizgen.com/>.

### RNase Decontamination

MERFISH measurements are sensitive to RNase activity. RNase contamination of any materials or reagents will degrade data quality.

Samples should be prepared in an area decontaminated with RNaseZap solution.

It is recommended to use RNase-free disposables, e.g., RNase-free media bottles (VWR PN: 82051-594) for preparing buffers.

### MERSCOPE Slide Handling

MERSCOPE Slides are fragile, handle with care. MERSCOPE Slides should be handled with tweezers, positioned to minimize the potential of touching the sample.

### MERSCOPE Flow Chamber Component Cleaning and Storage

Refer to [Load – MERSCOPE Flow Chamber](#) for flow chamber component cleaning operations as part of normal operations.

Refer to [Clean](#) and [MERSCOPE Flow Chamber Idle Procedure](#) for flow chamber cleaning operations in preparation for an idle period.

### MERSCOPE Codebooks

Gene panel-specific MERSCOPE Codebooks are CSV files that can be imported from local storage or the Vizgen Cloud. The applicable MERSCOPE Codebook must be imported prior to initiating an experiment.

The MERSCOPE Instrument scans the MERSCOPE Imaging Cartridge barcode for compatibility with the selected MERSCOPE Codebook after inserting the activated imaging cartridge into the instrument.

## Safety

Safe laboratory practices should be followed at all times.

Discard used MERSCOPE Slides per applicable institutional hazardous waste procedures.

The MERSCOPE Imaging Cartridge contains hazardous material and should be discarded per the MERSCOPE Imaging Cartridge Safety Data Sheet and applicable institutional hazardous waste procedures.

The MERSCOPE Instrument waste container contents should also be discarded per the MERSCOPE Imaging Cartridge Safety Data Sheet and applicable institutional hazardous waste procedures.

Safety Data Sheets for [Vizgen Materials](https://vizgen.com/) are available online at <https://vizgen.com/>.

## FRONT PANEL LED INDICATORS

### MERSCOPE Imaging Box Front Panel LED Indicator

When the MERSCOPE Instrument is on, the instrument status is displayed on the MERSCOPE Imaging Box front panel LED indicator.

Green	Progressing	Maintenance or status check running
Green	Constant on	Ready for use, no operations running
Green	Constant on	Configuring the experiment
White	Ramping	Experiment running
White	Breathing	Experiment completed
Blue	Breathing	Post-acquisition processing is complete, and no other processes are running
Red	Constant on	Not ready for use, but no immediate user attention needed
Red	Blinking	Major error requiring user attention (e.g., experiment error, power went off, program error)

### Waste Container LED Indicator

Green	Constant on	Waste container status OK
Red	Blinking	Waste container needs to be emptied

## ERRORS AND WARNINGS

### Cannot Import the Codebook

This error is received if the MERSCOPE Codebook file name is not in the correct file name format (*codebook\_0\_panelname\_panellID.csv*). Update the file name or select a different file and try again.

This error is received if the MERSCOPE Codebook file name extension is something other than .csv. Only files with .csv extension are allowed. Update the file name extension or select a different file and try again.

This error is received if no genes are detected in the MERSCOPE Codebook file. It is not possible to import a file with 0 genes. Update the file or select a different file and try again.

### Failure to Read the Barcode

This error is received if there is liquid on the barcode on the MERSCOPE Imaging Cartridge and/or the barcode reader in the MERSCOPE Imaging Box. Remove the imaging cartridge from the instrument, dry the barcode and the barcode reader, and reinsert the imaging cartridge. Click Scan Barcode to try barcode scanning again. If the MERSCOPE Instrument is unable to read the barcode, the barcode number may be entered manually.

### The Barcode Was Not Recognized

This error is received if the MERSCOPE Imaging Cartridge has been used already. It is necessary to insert a new imaging cartridge.

This error is received if there is an error in entering the barcode manually. Do not include any dashes or spaces in the number.

This error is received if the instrument software is out of date. Update the software per the instructions.

### The Inserted Imaging Cartridge Does Not Match the Codebook

This error is received if the selected MERSCOPE Codebook requires more bits than available in the inserted MERSCOPE Imaging Cartridge. If the codebook is correct but the imaging cartridge is incompatible, thaw and activate a new imaging cartridge.

### The Imaging Cartridge Has Expired

This error is received if the MERSCOPE Imaging Cartridge inserted has expired. It is possible to proceed, however the experiment may be compromised.

### High-Magnification Objective Focusing Quality is Insufficient

This error is received if the immersion oil on the high-magnification objective is in insufficient quantity or there are air bubbles in the immersion oil. Click **Try Again** to clean the high-magnification objective and repeat the immersion oil application. If the error persists, contact Vizgen Support ([support@vizgen.com](mailto:support@vizgen.com)).

### Update Failed

This error is received if a software update fails. It is possible to try again or try to update the software later.

### Low Liquid Flow Rate Detected When Washing Fluidic Lines

This error is received if the wash fluid is low. If the error persists, contact Vizgen Support ([support@vizgen.com](mailto:support@vizgen.com)).

### Waste Container Not Empty

This error is received if the waste container is too full (overweight). Best practice is to empty the waste container after each wash cycle or before starting an experiment.

### Out of Disk Space

This error is received if there is insufficient disk space on the MERSCOPE Instrument Computer and/or MERSCOPE Analysis Computer to run/process an experiment. It is necessary to delete/remove old experiment files to proceed with a new experiment/image processing analysis.

### Connection Lost

This error is received if the MERSCOPE Instrument Computer loses connection with the MERSCOPE Analysis Computer. It will not be possible to run an experiment until the connection is restored. Check the connections. If the error persists, contact Vizgen Support ([support@vizgen.com](mailto:support@vizgen.com)).

This error is received if there is a problem with the hardware on the MERSCOPE Instrument. If the error persists, contact Vizgen Support ([support@vizgen.com](mailto:support@vizgen.com)).

Although power cycling the MERSCOPE Instrument and the MERSCOPE Analysis Computer may help with restoring connections, **DO NOT** power cycle the MERSCOPE Analysis Computer while image processing analysis is in process.



## TROUBLESHOOTING

### Air Bubbles Remain in the MERSCOPE Flow Chamber After Wetting

It is possible to pull more liquid to try and remove air bubbles. The user interface leads users through this process. It is only possible to pull more liquid twice.

If air bubbles are still visible after pulling more liquid once, ensure the fluid connections are correctly assembled and tightly closed before pulling liquid for a second time.

### Air Bubbles Remain in the MERSCOPE Flow Chamber After 2x Wetting

Contact Vizgen Support ([support@vizgen.com](mailto:support@vizgen.com)). If the air bubbles are small, it may be possible to proceed with an experiment. However, the experiment may be compromised.

### Air Bubbles in the MERSCOPE Flow Chamber Input Line After Priming

If the input line is not debubbled at least up to the Fluidic Line Connector during priming, which may be a consequence of a clogged fluidic line or a faulty MERSCOPE Imaging Cartridge, contact Vizgen Support ([support@vizgen.com](mailto:support@vizgen.com)).

### Incompletely Thawed MERSCOPE Imaging Cartridge

It is essential to fully thaw the MERSCOPE Imaging Cartridge prior to activation, layering with mineral oil, and inserting into the MERSCOPE Instrument. An incompletely thawed imaging cartridge will not perform as expected and the experiment and/or the MERSCOPE Instrument may be compromised.

### MERSCOPE Imaging Cartridge Not Activated Before Insertion into the MERSCOPE Instrument

It is highly recommended to activate the MERSCOPE Imaging Cartridge prior to insertion in the MERSCOPE Instrument. However, if forgotten, it is possible to pierce the Cartridge Activate Port, insert the Imaging Activation Mix, and then layer the mineral oil while the imaging cartridge is in the instrument. If the imaging cartridge lid has been closed before activation, other reagent access points in the imaging cartridge foil will be punctured (by the imaging cartridge lid) – ensure no material/liquid is dropped into these other reagent access points. **DO NOT** invert the imaging cartridge after the activation port has been punctured and/or the imaging cartridge lid has been closed.

### Mineral Oil Not Layered into a MERSCOPE Imaging Cartridge Before Barcode Reading

It is highly recommended to layer the mineral oil into the MERSCOPE Imaging Cartridge prior to insertion in the MERSCOPE Instrument. However, if forgotten, it is possible to layer the mineral oil while the activated imaging cartridge is in the instrument (as long as the user has not proceeded to barcode reading, at which point the experiment is initiated and the imaging cartridge lid is locked). If the imaging cartridge lid has been closed before activation, other reagent access points in the imaging cartridge foil will be punctured (by the imaging cartridge

lid) – ensure no material/liquid is dropped into these other reagent access points. **DO NOT** invert the imaging cartridge after the activation port has been punctured and/or the imaging cartridge lid has been closed.

### MERSCOPE Imaging Cartridge Not Activated Before Initiating an Experiment

It is essential to activate the MERSCOPE Imaging Cartridge (and layer mineral oil) prior to initiating an experiment. If users forget to activate the imaging cartridge, the experiment will fail. The experiment should be aborted if users realize they have forgotten to activate the imaging cartridge.

### Mineral Oil Not Layered into an Activated MERSCOPE Imaging Cartridge Before Initiating an Experiment

It is essential to layer mineral oil into the activated MERSCOPE Imaging Cartridge prior to initiating an experiment. If users forget to layer mineral oil, they may proceed but the experiment may be compromised.

### MERSCOPE Imaging Cartridge Thawed but Activation and Experiment Cannot Be Started for Some Time

It is highly recommended to work with a MERSCOPE Imaging Cartridge directly after thawing. Contact Vizgen Support ([support@vizgen.com](mailto:support@vizgen.com)) if the imaging cartridge is thawed but an experiment cannot be started for some time.

### MERSCOPE Flow Chamber Aqueduct Caught in Base When Trying to Disassemble

If the Aqueduct of the Flow Chamber sticks when trying to remove it from the Base, ensure the Top is removed and then place the Base, MERSCOPE Slide, Gasket, and Aqueduct in a container with nuclease-free water up to the level where the Aqueduct meets the Base, and then place the container in ultrasonic water bath for 5 min before trying to remove the Aqueduct.

### Abort Experiment

If the experiment must be aborted, click **Abort Experiment** in the top right-hand corner. If the experiment is aborted, all data will be corrupted, and the information will be lost. Reasons users may need to abort an experiment include:

- Realization that experimental setup was incorrect.
- Realization that sample preparation was incorrect.
- Realization that the MERSCOPE Imaging Cartridge was not activated prior to insertion in the instrument.

After aborting an experiment, contact Vizgen Support ([support@vizgen.com](mailto:support@vizgen.com)) prior to removing the MERSCOPE Imaging Cartridge for advice on cleaning the instrument.

The MERSCOPE Imaging Cartridge may not be able to be reused after aborting an experiment. Contact Vizgen Support ([support@vizgen.com](mailto:support@vizgen.com)) for advice.

## APPENDIX A: SAFETY DOCUMENTATION

### A. Intended use of equipment

The MERSCOPE Instrument is intended for research use only. The MERSCOPE Instrument enables massively multiplexed, error-robust, single-cell in situ transcriptomic imaging. The MERSCOPE Instrument integrates high-resolution imaging, fluidics, and image processing into automated hardware to deliver precise measurements.

### B. Technical specification

MERSCOPE Instrument	Feature	Specification
	Sample Throughput	1 cm <sup>2</sup> of tissue per instrument run
	Resolution	60x oil immersion; 1.4 NA objective
	Lateral Resolution	100 nm pixel size
	Slide Capacity	Single MERSCOPE slide
	Multiplexing Capabilities	Up to 500-plex with current chemistry
	Imaging Camera	Back-thinned cooled sCMOS camera with 2048 x 2048 pixel sensor
	Illumination	Five laser lines, 405 nm, 475 nm, 545 nm, 635 nm, and 750 nm
	On Instrument Data Storage Capacity	16 TB
	Long Term Data Storage	Customer-provided long-term storage
	Instrument Dimensions	See figures below
	Power Source	120 V
	Automated Image Processing	Transcript decoding and cell segmentation



**C. Name and address of the manufacturer or supplier from whom technical assistance may be obtained**

Vizgen Inc.  
 61 Moulton Street  
 Cambridge MA, USA, 02138

**D. Information to mitigate risks found in the risk assessment**

Regarding the risks of using the MERSCOPE Instrument, there are no serviceable components, subsystems inside the system, all services shall be conducted by qualified Vizgen Service Engineers.

**E. Instructions for lifting and carrying**

Only authorized Vizgen representatives should uncrate and install the MERSCOPE Instrument. Mishandling of the instrument can affect the alignment or damage instrument components.

Do not relocate the instrument after installation and preparation. Moving the instrument improperly can affect optical alignment and compromise data integrity. If the instrument must be relocated, contact Vizgen Support ([support@vizgen.com](mailto:support@vizgen.com)).

Uncrating or moving an instrument by anyone other than an authorized Vizgen representative will void the warranty.

**F. Equipment ratings**

I. MERSCOPE Instrument supply voltage and power requirements

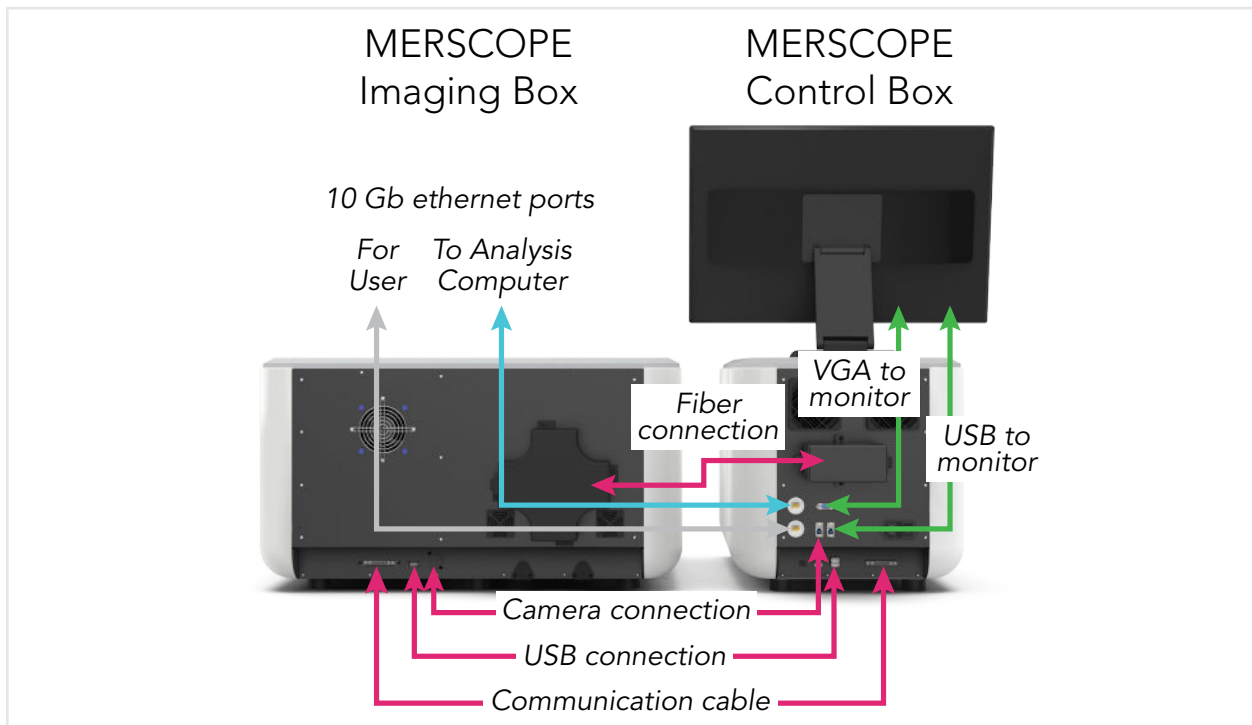
A maximum of 1500 W is required for the MERSCOPE Instrument, MERSCOPE Analysis Computer, and the instrument monitor.

Instrument Power Requirements	Specification
Line Voltage	100-240VAC – 50/60HZ
Power Supply Rating	650 W

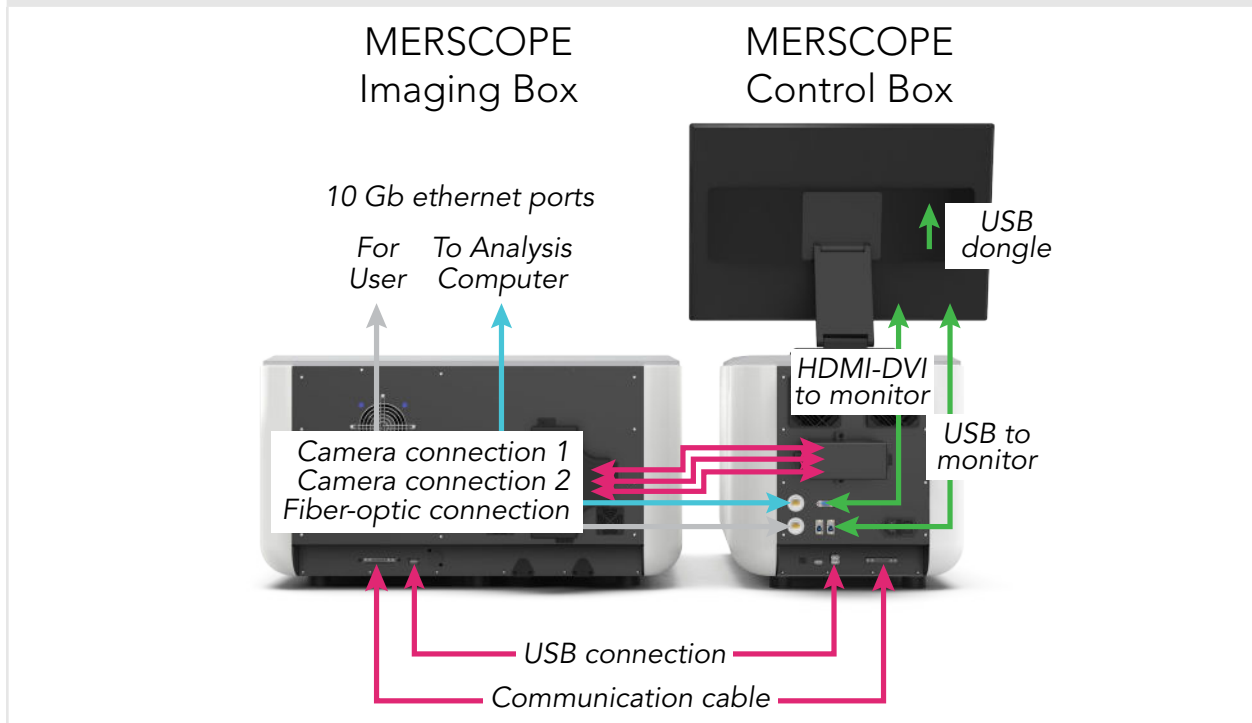
Analysis Computer Power Requirements	Specification
Line Voltage	100-240VAC – 50/60HZ
Power Supply Rating	850 W

Monitor Requirements	Specification
Line Voltage	100-240VAC – 50/60HZ
Power Supply Rating	28 W Typical (<1 W standby)

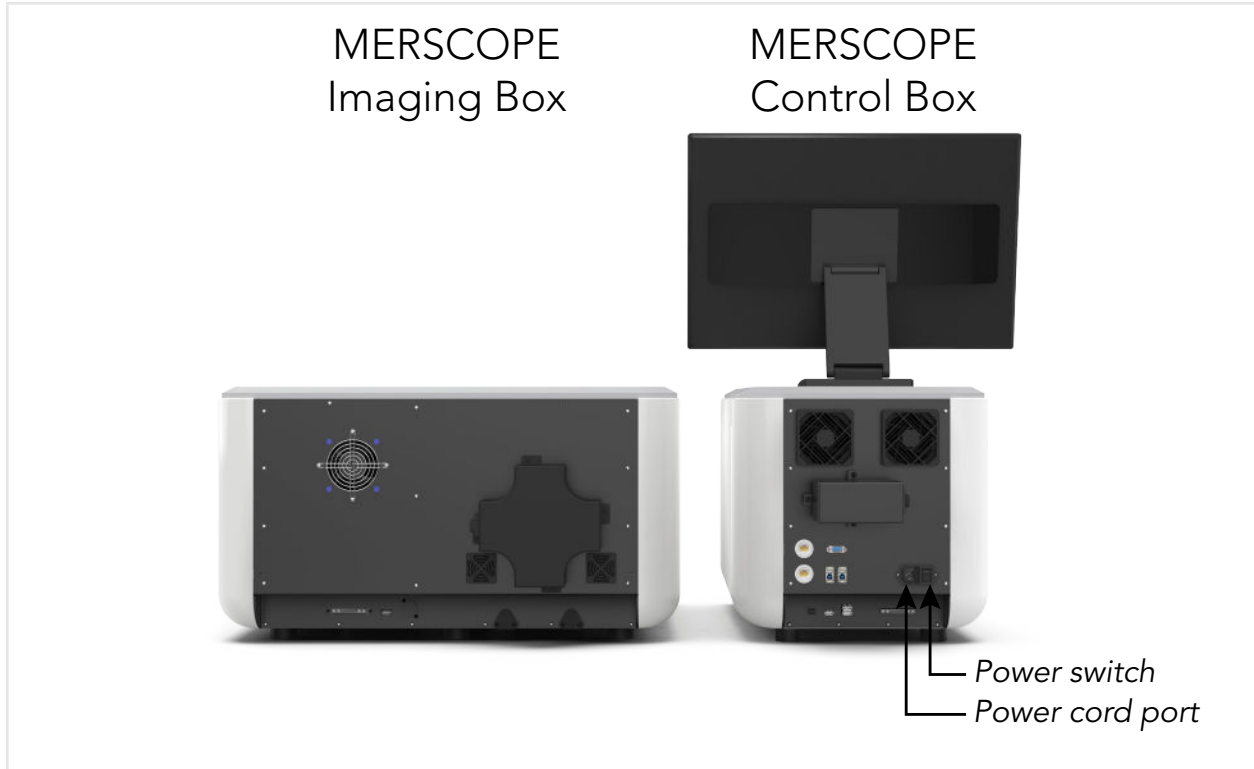
II. A description of all input and output connections



Instruments VMSC03501 and below.



Instruments VMSC03601 and above.



### III. Environmental conditions

Element	Specification
Operating Environment	Indoor use only.
Operation Altitude	2000 meters above sea level.
Temperature	Maintain a laboratory temperature of 19°C to 25°C. During a run, do not allow the ambient temperature to exceed 32°C.
Humidity	Maintain a non-condensing relative humidity between 20 and 80%.
Air Quality	Keep the instrument away from sources of dust. For indoor use only.
Vibration	Avoid intermittent shocks or disturbances near the instrument.

A user-supplied uninterruptible power supply (UPS) is highly recommended. Vizgen is not responsible for runs affected by interrupted power regardless of whether the MERSCOPE Instrument and MERSCOPE Analysis Computer are connected to a UPS. Standard generator-backed power is often not uninterruptible, and a brief power outage is typical before power resumes.

Consult with an institutional facility manager to obtain a UPS that complies with local standards.

Specification	Japan	North America	International
Description	APC Smart-UPS 2200 LCD 100V (Japan)	APC Smart-UPS 2200VA, Tower, LCD 120V with SmartConnect Port	APC Smart-UPS 2200VA, Tower, LCD 230V with SmartConnect Port
Part Number	SMT2200J	SMT2200C	SMT2200IC
Maximum Output Capacity	1.98 kWatts / 2.2 kVA	1.92 kWatts / 1.92 kVA	1.98 kWatts / 2.2 kVA
Input Voltage (nominal)	100 VAC	120 VAC	230 VAC
Input Frequency	50/60 Hz	50/60 Hz	50/60 Hz
Input Connection	NEMA L5-30P	NEMA 5-20P	IEC 320 C20, Schuko CEE 7 / EU1-16P
Dimensions (H x W x D)	17 x 8 x 22 in 43 x 20 x 56 cm	17 x 8 x 22 in 43 x 20 x 56 cm	17 x 8 x 22 in 43 x 20 x 56 cm
Weight	123 lb 56 kg	112 lb 51 kg	110 lb 50 kg
Typical Run Time (1200 W)	Approx. 17 min	Approx. 20 min	Approx. 19 min



The MERSCOPE Instrument is equipped with an international standard IEC 60320 C14 receptacle and is shipped with a region-specific power cord. Hazardous voltages are removed from the instrument only when the power cord is disconnected from the AC power source. To obtain equivalent receptacles or power cords that comply with local standards, contact Vizgen Support ([support@vizgen.com](mailto:support@vizgen.com)).

### **G. Equipment installation**

For MERSCOPE Instrument installation and operation requirements, review the *MERSCOPE Instrument Site Preparation Guide*, Document Number 91500001. Vizgen Service Engineers should also refer to the *MERSCOPE Instrument Installation Guide*, Document Number 91500002.

### **H. Equipment operation shall include**

See previous sections of the *MERSCOPE Instrument User Guide*, Document Number 91600001, for equipment operation instructions. For replacement of consumable materials, contact Vizgen Support ([support@vizgen.com](mailto:support@vizgen.com)). For legal notices regarding the MERSCOPE Instrument, see page 2 of the *MERSCOPE Instrument User Guide*.

### **I. Equipment maintenance and service**

- I. Refer to this manual and other documents provided by Vizgen on the proper usage and routine maintenance of the MERSCOPE Instrument. For instrument preventative maintenance and service, contact Vizgen Support ([support@vizgen.com](mailto:support@vizgen.com)). Vizgen Service Engineers should refer to the *MERSCOPE Instrument Service Guide*, Document 91600007.
- II. The instrument requires the use of the power cord supplied by Vizgen during the initial installation process.
- III. Replaceable fuses for the instrument: TA8 250VAC 5 x 20MM.
  - a. NOTE: As indicated in the label regarding the fuse (TAB8, 250VAC 5 x 20 mm) replacement, Vizgen recommends that the users contact Vizgen service team ([support@vizgen.com](mailto:support@vizgen.com)) to evaluate and replace the fuse indicated on the label.
- IV. The instrument does not require your on-site service personnel to maintain or service. All services shall be conducted by qualified Vizgen Service Engineers.